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PLASMA IRON AND THE TRANSPORT OF IRON IN THE ORGANISM

C.-B. LAURELL

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INTRODUCTION

In 1925, Fontès and Thivolle found that iron regularly occurred in horse plasma and that this iron was not haemoglobin-iron (65). This observation was confirmed and extended by Barkan (11) and by Henriques and Roche (86), who found that human plasma and plasma from various animals contains 50 to 220 μg per cent iron. The same year Warburg and Krebs (212) arrived at the same conclusion by the use of a catalytic method (211) for determination of μg-quantities of iron. This plasma iron fraction (easily-split-off iron of plasma, non-haemoglobin-plasma iron) is now generally called serum iron or plasma iron.

The central position of the plasma iron in the intermediate haemoglobin metabolism is evident from the fact that the iron is transported with the blood as plasma iron from the organs where the red cells are destroyed to the organs where new red cells are formed, and that the main part of the plasma iron forms a link in a narrow cyclic process (haemoglobin → plasma iron → haemoglobin) rather independent of absorption, excretion and storage. In normal individuals about 75 per cent of the plasma iron is derived from the catabolism of haemoglobin while the remaining 25 per cent is derived from the catabolism of myoglobin, haeme-catalysts, storage iron and absorbed iron. More than a thousand articles have appeared treating with different aspects of serum iron in relation to the intermediate iron metabolism of the body, but only those articles, which the author has considered to be of special importance, will be referred to here. A complete bibliography concerning papers on serum iron does not exist, but most of them can be found if the references in the numerous monographs (4, 9, 78, 80, 85, 113, 120, 150, 167, 182, 196, 198, 199, 202, 203) on serum iron are combined.

BIOCHEMICAL PART

Nature of serum iron

Barkan (11) showed that the iron in the serum was non-dialyzable at the pH of the blood, but after acidification iron appeared in the ultrafiltrate (12). The iron is thus protein bound and the iron-protein linkage is split on slight acidification. Starkenstein and Harvalik (185) fractionated sera to which iron salts had been added with ammonium sulphate and found that the iron was bound to the globulin fractions. With adsorption experiments Barkan showed (13) that the iron occurring in natural plasma was more firmly bound than the iron added to plasma. The precipitate obtained when saturating natural serum to 50 per cent with ammonium sulphate contained as much iron as the total serum. After partial electrophoretic separation of the serum proteins, Vahlquist (199) found that iron in serum was bound to both the albumins and globulins, but that the main part of the iron had a mobility corresponding to α- and β-globulins.

All these results were difficult to evaluate since very small traces of iron added as contaminants during the preparations could have influenced the results if iron added *in vitro* is not bound by serum in the same way as the iron originally existing in plasma, as indicated by Barkan (13). Yoshikawa *et al.* (218) therefore found it more satisfactory to work with natural plasma containing radioactive serum iron. They used plasma from dogs two hours after feeding the animals with radioactive iron. After saturation of the plasma to 50 per cent with ammonium sulphate, only about 15 per cent of the radioactive iron of the plasma was recovered in the precipitate, *i.e.*, in the globulin fraction. If the serum proteins were precipitated with trichloroacetic acid more than 90 per cent of the radioactive iron was recovered from the supernatant. When plasma was dialyzed against distilled water, about 5 per cent of the iron was found to be dialyzable. These results confirmed the earlier findings that iron in the serum is protein bound and acid soluble, but indicated that the iron in natural plasma is bound to some of the albumins.

Clinical experiments showed that the highest serum iron values obtained after peroral iron tolerance tests in healthy people was about 300 μg per cent (138) and that about the same values were obtained when ionized iron salts were injected intravenously. The subjects showed signs of intoxication if the intravenous iron injection was continued after this limit had been reached (207). This phenomenon made Holmberg propose the hypothesis that serum is only capable of forming a complex binding of iron up to a certain limit corresponding to about 300 μg per cent iron. Holmberg and Laurell (91) were able to show that the iron in the serum iron-protein complex at physiological pH does not react with $\alpha\alpha_1$-dipyridyl (a strong complex former for ferrous iron) in spite of the presence of sodium dithionite. After addition of ferrous salts to serum the added iron is bound, up to a certain concentration (saturation limit), in non-dipyridyl-reacting form. On the other hand, all iron added in excess over the saturation limit reacts with dipyridyl. The mean value for the saturation limit in healthy persons corresponds to the binding of about 315 μg iron per 100 ml. serum, *i.e.*, normally the iron binding component is saturated only to about 30 per cent with iron as the normal serum iron is about 100 μg per cent. They also showed that when ferrous iron is added to serum a change in serum colour appears from yellow to yellow-red, easily determined in a photometer at about 5000 Å. This change of colour is developed successively with the addition of increasing small amounts of iron and seems to reach its maximum at the saturation limit. The same observation was independently made by Schade and Caroline (169). Earlier they had observed a similar change in the colour after addition of iron to hen's egg white (168). The component responsible for the latter reaction was identified as conalbumin (169). Schade *et al.* investigated the various protein fractions obtained by ethanol fractionation of plasma according to Cohn *et al.*, and found that the colour change occurs only when iron salts are added to fraction IV:3,4 (43). That the salmon pink colour appearing after addition of iron was due to the formation of a specific iron-protein complex was proved by a sensitive microbiological method (168).

Further evidence for the existence of a specific iron-binding protein component in serum was obtained by the observation that iron added to serum above the iron-binding capacity of serum (saturation limit) is dialyzable and can be adsorbed to AlC γ (120).

Terminology

Synonyms for the iron-binding protein of plasma are *transferrin*, *siderophilin*, *β_1-metal combining (pseudo) globulin*, and *iron-binding component of plasma*.

Synonyms for *serum iron* are *plasma iron*, *Fe-transferrin*, and *acid-soluble*, *non-haemoglobin iron of plasma*.

Increased concentration of serum iron is called *hyperferraemia* or *hypersideraemia*, and decreased concentration of serum iron is called *hypoferraemia* or *hyposideraemia*.

The transferrin concentration of serum (plasma) is generally expressed indirectly as the capacity of serum to bind iron specifically. Names such as *total iron-binding capacity* (*TIBC*) and *saturation limit* have been used.

The *total iron-binding capacity* minus the iron actually bound (serum iron) has been called the *latent iron-binding capacity*, the *unsaturated iron-binding capacity* (UIBC), or the *unbound iron-binding capacity*. Some authors have unfortunately used the term iron-binding capacity which elsewhere is generally used as an abbreviation for total iron-binding capacity.

Transferrin, the iron-binding component of plasma

Schade and Caroline have shown that fraction IV:3,4 of human plasma contained the iron-binding component (169). Further subfractionation of serum later showed that the main part of the iron-binding component could be further purified and concentrated in fraction IV:7 (189). The iron-binding protein in iron-free state can be crystallized (112). The fractionation scheme of Cohn gives the iron-binding component in a mainly iron-free state. Laurell (119) saturated pig's plasma with iron before the fractionation, as the component is more stable when saturated with iron. Crystallization of the Fe-transferrin complex can easily be brought about by addition of ethanol to a salt-free solution of the pure protein at a pH about 6 until the first turbidity appears at 0°C. (122).

Iron-free transferrin shows no characteristic absorption of light in the visible region of the spectrum but, when saturated with iron, it has a strong red-orange colour with broad absorption maximum at 4700 Å (92, 188). In the ultraviolet, transferrin and Fe-transferrin have an absorption maximum at 2800 Å as do other proteins (188). The molecular weight of the protein is about 90,000 (188) (88,000 (119)). In electrophoresis (pH 7.5–8.8), transferrin moves with the group of β-globulins. It has therefore been called the β_1-metal combining globulin. Determinations of the iso-electric point have given varying results (5.9 (188), 4.4 (119)). The first value was obtained on analysis of human material, the latter on a preparation from pig. Moreover, different buffers and ionic strengths have been used. The investigated human protein was probably iron-free, but the protein from pig was saturated with iron. These differences may explain the diverging results. It has to be stressed that the mobility of this protein is markedly influenced by the kind and concentration of the inorganic ions in the buffer solution used. Further experimental work is necessary to elucidate this fact. The term "metal-combining *globulin*" used by Cohn is somewhat misleading as this protein is soluble in a solution half saturated with ammonium sulphate (119) and thus follows the albumins in this respect. Fe-transferrin is more soluble than iron-free transferrin on the alkaline side of its iso-electric point (44, 112). Solubility studies are presented by Koechlin (112).

Transferrin does not contain lipids (188). It seems to contain 1.8 per cent carbohydrate (188), but conalbumin, the corresponding protein from hen's egg white (213), is free of carbohydrate. The carbohydrate component is thus probably of no importance for the iron-binding capacity of transferrin. The nitrogen content is 14.7 per cent (112, 188).

Each molecule of transferrin can bind two atoms of iron (112, 119, 188). According to the present experimental facts, the two iron-binding groups of transferrin have such a position on the protein surface that there is no interaction between them, *i.e.*, that the energy content of the both Fe-protein linkages is the same (121).

The reaction between iron and transferrin is dependent on pH (63, 120, 188). If iron is added to a salt-free solution of transferrin at neutral or slightly alkaline reaction the added iron is bound to transferrin as is evident from the appearance of the typical change of colour. For complete binding of the iron practically no excess of iron has to be added. On acidification of the solution with dilute hydrochloric acid, the colour disappears at pH 4 and the iron can be dialyzed away. The dissociation of the complex and the loss of colour are readily reversed by readjusting the system to pH 7 (63, 120, 188). A partial dissociation of the iron-transferrin complex can already be observed at pH 7 in the presence of serum (63, 120) or of phosphate ions (63), which means that the iron-transferrin complex is not completely undissociated within the physiological range of pH. The affinity constant has been determined to be approximately 10^7 at pH 7 (45). The data of the dissociation equilibrium are difficult to measure as the iron is rapidly hydrolyzed at neutral or slightly acid reaction. The iron-transferrin complex is stable up to pH 10, but is irreversibly destroyed at higher pH (63).

Transferrin can be acidified to pH 1.5 and the iron-binding capacity is nevertheless retained after neutralization (63), but it seems doubtful whether transferrin can be crystallized again after such a rough treatment since the crystallizability of conalbumin is lost if pHs below 4 are used during the preparation (213).

A yellow colour (light absorption maximum 4350 Å) appears on addition of copper ions to a metal-free solution of transferrin (92, 112, 188), but a more alkaline reaction is necessary for a complete binding of copper than for iron (188). That copper is bound by the same groups as iron is evident from the fact that the copper can be displaced by an equivalent amount of iron (92, 188). Within the physiological range of pH the copper is loosely bound to transferrin and the affinity of transferrin for copper is not higher than that of other serum proteins for copper (92). The main part of the serum copper is bound to an α_2-globulin (93). So far, no facts favour Cohn's hypothesis (43) that transferrin is of any importance for the copper transportation in the body, and this is also probably true for zinc, which is even more loosely bound than copper to transferrin (92, 188).

The same amount of iron is bound by transferrin (and serum) when either ferrous or ferric iron is added, and the change in absorption spectra obtained are identical (63, 64, 88). The reaction is more rapid with ferrous iron. The slower reaction with ferric iron may depend upon the lower solubility and the more rapid hydrolysis of ferric iron at neutral pH, since the autoxidation of ferrous iron was found to be a prerequisite to the binding (112). The result of magnetic susceptibility measurements performed by Michaelis on the iron-transferrin complex indicated that the iron is in the ferric state and that the bond is ionic (188).

It has been claimed that carbon dioxide (or bicarbonate) is necessary for the reaction between iron and transferrin (63, 64, 170). Based on comparisons between the complex iron compounds of hydroxylamine and carbon dioxide, and between iron and cyclic hydroxamic acids and the complexes formed between iron, carbon dioxide and transferrin, Fiala and Burk (63, 64) suggested that the groups responsible for the bond between iron and transferrin or conalbumin were hydroxamic acid groups. On the other hand, Fraenkel-Conrat (66, 67) found that bicarbonate is not necessary for the development of the coloured iron-transferrin complex, and he was unable to demonstrate the presence of hydroxylamino or alkylhydroxylamino groups of any kind in conalbumin and transferrin, even though sufficiently sensitive methods were available. The nature of the groups responsible for the high affinity of transferrin for iron is thus still obscure.

Other iron-protein compounds than transferrin in blood, of proposed importance for iron transportation

During the recent years some experiments with salting out and dialysis have been published (125, 126, 142, 143, 157, 191, 193), on the results of which the authors base the opinion that transferrin is not the only plasma protein of importance for the transportation of iron. Therefore Wallenius (209) reinvestigated the distribution of plasma iron between the different serum proteins by means of combined paper electrophoresis and autoradiography. He found no support for the assumption that more than one component is responsible for iron transportation. The activity was concentrated in the β-globulin, *i.e.*, in transferrin independently of whether the radioactive iron was given perorally or parenterally.

The deviating results of the salting out experiments can possibly be explained by the fact that addition of ammonium sulphate to unbuffered protein solutions will result in a pH of about 4–5.5, a range within which the natural serum-iron complex is partly dissociated. The conflicting results obtained with dialyzing experiments may be explained by the rapid hydrolysis of iron in solutions of pH higher than 5 in the absence of complex formers for iron.

The small amounts of *haemoglobin* found in plasma from normal subjects is probably a preparative artefact.

The iron in methaemalbumin (133) is not so firmly bound as that in haemoglobin, but since methaemalbumin in measurable quantities is only found in pathological sera (*e.g.*, from patients during haemolytic crisis) it is presumably an expression of an abnormal haemoglobin metabolism and probably does not play any role in normal haemoglobin catabolism. The plasma iron fraction observed by Plumier and Lambrechts (155) may possibly be methaemalbumin.

From a series of fractionated serum-iron determinations, Vannotti and Delachaux (202, 203) suggested that four different serum-iron fractions (A, B, C and D) occur in normal plasma. Their fractions A and B correspond to the usual serum iron fraction (transferrin-bound iron). Fraction C is obscure and fraction D seems to be haemoglobin iron. Their methods have, however, been severely criticized. (For literature, see Kooyman (113) and Hemmeler (85)). Normal plasma does not contain *ferritin* (the iron-storage protein) which has only been found in plasma in irreversible shock (72). *Red blood cells.* Barkan (12) adopted the same method for determination of acid-soluble iron in red blood cells as he had previously used for determination of plasma iron. The iron fraction obtained has since then been called "easily split off" blood iron. It was later (14) separated into two fractions E and E^1. Fraction E has been shown to be an artefact (74, 129). Grinstein and Moore (74) made a thorough study of the easily split-off iron with the aid of radio-iron and their data indicated that "easily split-off" blood iron as defined by Barkan *et al.* is an artefact and originates entirely or almost entirely from intact haemoglobin. Agner (1) has demonstrated *ferritin* in red blood cells from horse. If ferritin also exists in human erythrocytes (which is unproven), it will contribute to fraction E^1. Red blood cells contain no iron-free transferrin (120) but the possibility that they contain small amounts of Fe-transferrin can at the present not be excluded.

Nobody has investigated whether the *white blood cells* have any function in iron transportation or haemoglobin break down, in spite of the fact that leucocytes have a relatively high iron content.

Iron exchange between plasma and blood corpuscles

Barkan *et al.* (14, 15) showed an increase in serum iron when whole blood was left standing at 37°C. for 24 hours. In contrast thereto, Moore *et al.* (135) found no such increase. Lambrecht and Plumier (116) found an increase in serum iron after incubation, but this increase was highly dependent on the anticoagulants used and the degree of haemolysis during the incubation. There are no reasons to assume a passage of iron from vital red blood cells to plasma, since the haemoglobin degradation in the normal red blood cells seems to be negligible (74).

With radioactive iron it has been shown (130, 210) that *reticulocytes*, but not *mature erythrocytes*, can assimilate iron from plasma. The assimilation proceeds more rapidly when the cells are mixed with inorganic iron than with Fe-transferrin.

Taken as a whole, the exchange of iron between plasma and red blood cells seems to be negligible when compared with the rapid serum iron turnover.

Transferrin-bound iron also occurs in the *lymph* but in lower concentration than in the plasma (138). Preliminary experiments indicate that the transferrin concentration of the intestinal lymph (rat) is about one third of that of plasma (122). The transportation of iron via the lymphatic vessels during iron absorption seems to be negligible compared with the plasma transport (59).

Methods for determination of serum iron

A critical review of the methods used for serum iron determination (2, 25, 29 53, 78, 96, 110, 197, 199) has been written by Hemmeler (85) and only a few remarks on some discrepancies will be made here.

Most methods used are modifications of Heilmeyer and Ploetner's method (78). The proteins of the serum or the plasma are precipitated at acid reaction, the ferric iron in the filtrate is reduced by a suitable reagent to ferrous iron which is determined colorimetrically after addition of substances forming coloured complex iron compounds. Most modifications using this principle seem to be relatively reliable.

When analyzing sera from haemolyzed blood, incubation of the serum at a strong acid reaction for a short time gives more reliable values than incubation at a weak acid reaction for several hours.

There has been much discussion about the optimal pH for the determination of iron with phenantroline (85, 199). This discussion has been confusing since the main problem has not been stressed. The ferrous iron-phenantroline complex is stable with the same specific light absorption within the pH range 1.3–9. Therefore the pH suitable for the determination depends only on the reducing substances used. Hydrazin sulphate (197), thioglycollic acid (110) and sodium dithionite (91) rapidly reduce ferric iron in solutions of pH between 4.5 to 4.9. Hydroquinone is unsuitable for general use since it is only practicable within a narrow pH range about 2 (199). Neutralisation of the acid serum extract to the pH wanted is most simply effected by addition of a suitable amount of a concentrated solution of sodium acetate (96, 197) or with a mixture of concentrated sodium acetate and ammonium hydroxide, to avoid titration of each sample as is described in most methods.

Most methods used for the determination of serum iron are not quite reliable when used to estimate the iron concentration of serum containing colloidal ferric oxides, *e.g.*, during the hours following an intravenous injection of saccharated ferric oxide. No critical study has been presented concerning this simple methodological problem.

Determination of the transferrin concentration of plasma (saturation limit, iron-binding capacity)

Two fundamentally different methods have been worked out for the determination of the transferrin concentration in serum. One is indirect and founded on the estimation of the iron-binding capacity of serum, *i.e.*, the amount of iron-reactive groups in serum with the same affinity for iron as those of transferrin (91, 169), while the other is direct and founded on the precipitation of the iron-binding protein with a specific antibody (101). The latter is complicated, but of great interest since with the aid of this method it has been definitely established (101) that changes in the iron-binding capacity of serum always run parallel with changes in the concentration of transferrin. Decrease of the iron-binding capacity of plasma thus depends upon a decrease of the concentration of transferrin and not upon blocking or destruction of the iron-binding groups of transferrin.

The total iron-binding capacity of serum (TIBC) can be determined in two different ways. One is founded on the fact that iron, when added to serum in excess of the iron-binding capacity, reacts with phenantroline (or $\alpha\alpha_1$-dipyridyl) after addition of a small amount of sodium dithionite (91, 120). The other is founded on the assumption that the whole colour-change of serum that occurs after addition of iron depends on the formation of the coloured iron-transferrin complex (169). The unsaturated iron-binding capacity (UIBC) can be computed from the change of the serum colour since the specific absorption of the iron-transferrin is known. The TIBC is then obtained by addition of the values for UIBC and for serum iron. This last mentioned method (169) has been employed by most investigators with minor modifications (37, 111, 158, 188) since it is extremely simple. It has, however, certain limitations. Lipaemic and icteric sera cannot be analyzed (37, 158). False values are obtained if any coloured substance exists in serum which can be reduced by the ferrous iron generally used for the determination. That this simple method is not quite reliable even with normal plasma is evident from the values for TIBC recorded by some authors (18, 90, 180, 184) who have determined the TIBC of serum from the same person at short intervals and found variations which must be considered as extremely unlikely.

The phenantroline method (120) has not the limitations given above, but perhaps others. Concerning this method it has to be stressed that it is necessary to use sodium dithionite of highest quality (Merck pro analysi) and that the serum containing phenantroline and phenantroline iron has to be added drop by drop to the hydrochloric acid with cautious shaking to effect a rapid decrease in pH of the serum added, since the iron liberated from the Fe-transferrin of the serum by the acid otherwise may be bound by phenantroline which results in a too low value for the TIBC of serum.

PHYSIOLOGICAL PART

Serum iron and transferrin in normal subjects

Adults. As mentioned later the serum iron shows pronounced diurnal variations with higher morning than evening values. Not every investigator has taken this into consideration when the materials have been gathered for analysis of the normal variation of serum iron. In general, however, the materials consist of samples gathered in the morning. The mean values recorded by different authors (85) show relatively good agreement and vary for adult men between 118 and 142 μg per cent and for adult women between 90 and 123 μg per cent. The sex difference is statistically significant (199). The statistical distribution has not been found quite binomial by most authors and the scattering differs considerably from material to material. From a practical point of view it can be stated that values above 220 μg per cent and below 70 μg per cent in men and above 200 μg per cent and below 60 μg per cent in women are rarely found, when blood is analyzed which has been withdrawn in the morning from fasting, thoroughly rested individuals.

The reason for the sex difference has been actively discussed, but no agreement has been reached (85, 200, 201). It has to be pointed out, however, that the mean value for the depot-iron fraction is greater per kg body weight in men than in women (117, 165). The contrary is found in rats (166, 215). It would therefore be of interest to investigate whether the mean value of serum iron in female rats is higher than in male rats.

The mean recorded values of the *transferrin concentration* of plasma, expressed as the total iron-binding capacity, vary between 300 and 360 μg per cent (37, 91, 120, 158, 204). The greatest material (100 cases) so far studied showed a mean value of 315 μg per cent (the standard deviation for a single observation was determined to be 33 μg per cent) (120). This value corresponds to a concentration of about 2.5 g transferrin per liter plasma. No statistically significant sex difference has so far been observed, but Cartwright (37) has found somewhat higher values in women than in men.

Children. The serum iron content of umbilical blood (about 150 μg per cent) is somewhat higher than in blood of normal individuals and of the mothers (50, 85, 89, 120, 199, 216). During the first two days of life a hypoferraemia of about 50 μg per cent develops. During the following 6 days there is a steady increase of the values to about 120 μg per cent. A new decline then begins and at an age of 8 months to 2 years a new minimum is reached with 50–60 μg per cent serum iron. The values thereafter again increase slowly up to the puberty when the mean values of adults are reached (4, 5, 46, 196, 199). The sex difference first appears after puberty (200, 201). No clearcut variation in serum iron is seen among the different age groups of normal adults (41).

No systematic study on the iron-binding capacity of serum during infancy and childhood has been published. Laurell (120) found that serum from umbilical blood contained significantly lower amounts of transferrin (TIBC = 226 μg per cent) than serum from adults (TIBC = 315 μg per cent). Smith found higher iron binding capacity in children (aged 2½ to 10 years) than in adults (184).

The *diurnal variation* of the serum iron values was first observed by Vahlquist (199), and later analyzed by many authors (61, 76, 84, 97, 147, 177, 192, 208). Most authors find the serum iron values about 10 to 30 per cent higher in the morning than in the evening. Hemmeler (85) who has performed the greatest experimental work on this subject states that the diurnal rhythm with higher morning than evening values is valid only if the patients have slept during the night and that the increase of serum iron during the night is highly dependent on the duration of the sleep. The diurnal rhythm of the serum iron is found reversed in normal subjects working nights and sleeping during the day-time (85, 97, 177, 208).

No diurnal variation of the iron-binding capacity of serum has been observed (122).

With repeated determinations of serum iron in the same individual at intervals of *days, weeks* and *months*, some authors (78, 135, 182) have found only slight variations from time to time (30 μg per cent or less), others (85, 96, 113, 167) a considerably higher variation.

Repeated determinations of the TIBC in the same individual suggest that the transferrin concentration is rather constant over long periods in healthy individuals with normal blood status.

Seasonal variation is of little practical importance, as the small differences found (higher autumn than spring values) are not statistically significant (198, 199).

Meals. No common foodstuff contains so much available iron that the intake of meals can influence the serum iron (78, 182, 195), but as later shown, intake of absorbable iron compounds gives a sharp increase of the serum iron level.

Muscular work does not seem to influence the serum iron values (19, 55, 85).

The *menstrual cycle* is without influence on the serum iron values (25, 36, 97, 179). Dahl (51) and Powell (156) state, however, that the serum iron values are highest shortly before, and lowest during and immediately after menstruation.

Pregnancy. The extensive literature on the variations of the serum iron during pregnancy has been reviewed by Lundström (131) and Ventura (204). The results of earlier investigators diverge surprisingly, but authors of the more recent publications (50, 60, 89, 120, 131, 187, 204) agree that the serum iron values are unchanged during the first 20 weeks of pregnancy, after which a slight hypoferraemia develops. The lowest values are found during the two last months of pregnancy (the decrease is about 30 per cent). This decrease is greater than can be expected from the effect of the hydraemia of pregnancy.

During the hours following delivery a further decrease in serum iron appears and the normal serum iron level is not reached until some months after parturition (50, 144, 161, 199).

The total *iron-binding capacity* of plasma is unchanged during the first 4 months of pregnancy; thereafter increases considerably, and reaches its maximum during the last two months. The increase found is about 50 per cent (60, 89, 120, 204). Rath *et al.* found only a slight increase (159).

During the two months following delivery the transferring level is gradually normalized (60).

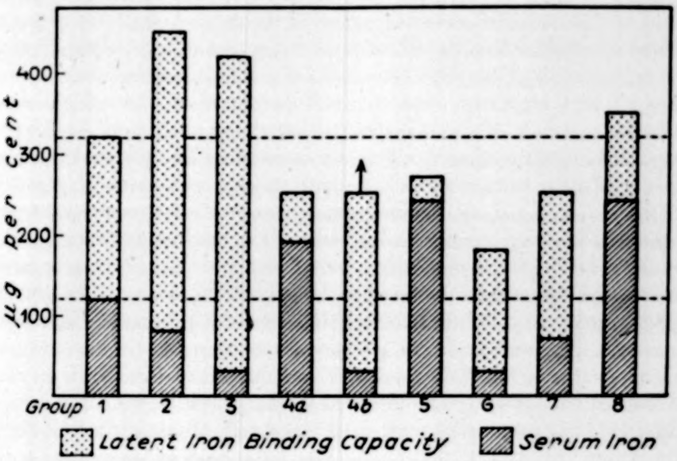

FIG. 1. Serum iron and total iron binding capacity of serum in various disturbances of iron metabolism.

1. Healthy adults (37, 120, 158).
2. Late pregnancy (60, 89, 120, 204).
3. Chronic iron deficiency (27, 28, 37, 78, 90, 103, 106, 120, 124, 158, 165, 183, 184); polycythaemia vera (28, 30, 36, 85, 95, 214).

4a. Haemolytic (8, 28, 36, 124, 153, 154, 171, 184), pernicious (30, 54, 120, 136, 158, 206, 207), aplastic (30, 36, 80, 120, 163, 199, 214), and myelophthisic (214), anaemias (fetus at parturition).

4b. Haemolytic and pernicious anaemias during remission.

5. Haemochromatosis (7, 10, 18, 44, 56, 68, 85, 100, 158) and transfusion haemosiderosis (158).
6. Acute infections (Hodgkin's disease) (34, 35, 37, 48, 57, 78, 80, 87, 120, 160).
7. Chronic infections (35, 37, 105, 109, 113, 120, 142, 147, 198), malignant tumours (85, 120), myelomatosis (120), uraemia (28, 36, 85, 120), leukaemias (23, 28, 36, 120, 158), cirrhosis of the liver (18, 120, 128), acute or subacute liver atrophy (18, 85).
8. Acute hepatitis (second to fifth week) (17, 20, 21, 26, 58, 83, 85, 120, 128, 181).

Serum iron and transferrin level in various diseases

The typical changes of serum iron and total iron-binding capacity of serum found in various diseases are schematically represented in figure 1. Groups 2 and 3 represent conditions with increased requirement for iron. These conditions are characterized by the following facts: i) the quotient between serum iron and latent iron-binding capacity of serum is considerably lower than normal; ii) ingested iron is utilized better than normally; iii) iron is mobilized from the iron depots. In most cases with iron deficiency the TIBC of blood is clearly increased as judged from direct determinations (37, 120, 158, 184) and from the degree of hyperferraemia attained after peroral iron tolerance tests (103, 164). In case of essential hypochromic anaemia, however, further studies on the TIBC of serum are necessary to elucidate whether this disease is accompanied by a disturbed regulation of the transferrin concentration of serum and the intermediate iron metabolism or whether it depends on poor utilization of ingested iron (82, 102, 190).

Groups 4 and 5 represent conditions with progressively increasing stores of iron. It is characteristic of these conditions that the quotient between serum iron and latent iron-binding capacity of serum is considerably higher than normal. During spontaneous remissions or following specific treatment of the diseases belonging to group 4, hypoferraemia develops and the TIBC increases (inversion of the quotient between serum iron and latent iron-binding capacity), and these changes are combined with a mobilization of iron from the storage organs.

Groups 6 and 7 represent the findings during infections and allied conditions. The hypoferraemia and the decreased TIBC are most pronounced during acute infections with high fever and during severe chronic diseases. The anaemia which often develops during these diseases does not, however, depend on an iron deficiency as earlier supposed. The retarded erythropoiesis seems to be caused by an inhibition of the maturation of the red blood cells at a stage before the incorporation of iron (34) and this anaemia is not influenced by peroral (172, 173) or intravenous iron therapy (114, 115). The hypoferraemia is supposed to depend on an increased accumulation of iron in the reticulo-endothelial system (38, 73, 80, 165). The form in which iron is bound by the reticulo-endothelial system in these diseases has not been explored. During the regression of the infections the iron is liberated and made available for haemoglobin synthesis at the same time as the transferrin concentration is normalized. A series of severe systemic diseases (malignant tumours, myelomatosis, etc.) show the same type of changes as found in infections, but less pronounced (Group 7).

The only disease which belongs to group 8 is acute hepatitis. Although the main storage organ for iron is attacked in this acute infection, this fact cannot explain the hyperferraemia sufficiently since there is no clear correlation between the severity of the disease and the degree of hyperferraemia. Marked hyperferraemia is often found in mild cases (85, 128), and in acute or subacute yellow atrophy hypoferraemia often develops after one or two weeks (18, 85). The reason for the hyperferraemia during acute hepatitis has been discussed, but no definite conclusion has been reached (85).

Effect of iron dosage

Iron given intravenously. A dose of iron salts corresponding to 5 to 10 mg Fe administered intravenously to human subjects may be sufficient to provoke slight toxic symptoms, especially when the iron is injected rapidly. The symptoms consist of flushing of the face, nasal stuffiness, sneezing, lacrimation, nausea, vomiting and paraesthesias in the arm in which the iron is injected (70). They disappear, however, rapidly. This toxic reaction seems to be caused by an abrupt increase of the iron ion activity of plasma, which normally is extremely low. The amount of iron tolerated during slow injection depends mainly on the amount of iron-free transferrin in the blood which can de-ionize the injected iron (91, 207). Patients with a small amount of iron-free transferrin in plasma (*e.g.*, pernicious anaemia, haemochromatosis etc.) show symptoms of intolerance after injection of a few milligrams of iron and patients with high amounts of iron-free transferrin (iron deficiency, pregnancy) can tolerate about 20 mg. If the injection of iron is continued after the appearance of toxic symptoms the main part of the injected iron rapidly leaves the circulation, and the serum iron values rise only slowly. In animal experiments 70–80 per cent of the iron injected beyond the iron-binding capacity of plasma disappears within a few minutes (141), and it is reasonable to assume that a rapid distribution of the ionized iron between the blood plasma and the extracellular tissue fluid takes place. The iron concentration of plasma obtained some minutes after injection of 10 mg of iron (17, 18, 198, 207) is a good expression of the total iron-binding capacity of plasma except in cases with abnormally high transferrin concentration of the plasma.

The decrease of the serum iron during the hours following the injection varies. In normal individuals the serum iron decreases 0–80 μg per cent during the first two hours after the injection, and the initial serum iron values are not reached until after five to ten hours. In patients with iron deficiency (207), with pernicious anaemia during remission (207), and with acute infections (34, 198) a more rapid decrease has generally been observed. The increased elimination rate in these conditions can be explained by the increased bone marrow activity or, in the case of infections, by the increased affinity of the reticulo-endothelial system for iron.

The total iron-binding capacity of serum is not influenced during the change of serum iron following an intravenous injection of iron (37).

Intravenous iron therapy. Heath *et al.* (77) found that 96 per cent of intravenously injected iron was utilized for haemoglobin synthesis, but the toxic reactions were too severe to warrant its clinical use. The toxicity of the iron preparations depends on the rate with which iron ions are liberated from them. Solutions of iron cacodylate ($Fe[(CH_3)_2As_2O_3]_3$) (127) or sodium-bis-($\alpha\gamma$-dioxy-$\beta\beta$-dimethylbutyrato)-ferrate (194) have been used in the clinic since they form iron ions more slowly than simple inorganic iron salts, but nevertheless the tolerated dose of these compounds has often been found insufficient for general clinical use. Ferritin-iron can, however, be injected in the amounts desired (daily injection of 100–200 mg. Fe) without toxic manifestations, but has the disadvantage of being antigenic when not prepared from human material (3). Saccharated ferric oxide (148, 149) seems to be a rather valuable therapeutic agent. Daily injections of 100 mg and more of iron in this form are generally well tolerated. It is rapidly taken up by the reticulo-endothelial system and in this way eliminated from the blood (40, 178). The urinary loss is less than 10 per cent of the dose (178). After intravenous administration of saccharated ferric oxide, the iron from this compound is so rapidly utilized that iron no longer need be the limiting factor in the blood regeneration of subjects with anaemias of iron-deficiency type (24, 94, 108). However, the rate of ionization does not exceed the rate at which ferritin formation and other iron ion detoxicating mechanisms of the body can prevent an increase of the iron ion activity in the tissues which causes toxic symptoms. The slow liberation of iron from the colloid is evident from clinical experiments; thus the plasma transferrin is not saturated with iron even 30 minutes after the intravenous injection of 100-200 mg (33, 90).

The high values for total iron-binding capacity of serum and the low values for serum iron in patients with iron deficiency are normalized during the course of intravenous treatment with saccharated ferric oxide (32, 90, 111).

Agner has developed another iron preparation in which the iron is linked to a soluble, high molecular carbohydrate, and built up in a manner similar to ferritin (9). Clinical experiments with this preparation have given results which seem to be as satisfactory as those obtained with various preparations of saccharated iron oxide (9).

No experiments have been published to determine whether the injected iron colloids circulate free or bound to protein in the plasma until they are taken up by the reticulo-endothelial system.

Iron administered perorally. The amount of iron absorbed from the intestinal tract in healthy people is regulated by the demand of the organism for iron. After oral loadings with large amounts of iron the serum iron level responds with a considerable increase and some iron is thus absorbed even in individuals who must be regarded as being in complete iron equilibrium. The serum iron curves after oral iron loadings are, however, difficult to interpret since the serum iron increase is influenced by different factors such as the rate of iron absorption, the rate of iron elimination from the blood, the plasma volume, the transferrin concentration, etc. The serum iron increase can thus not be used as a reliable expression for the amount of iron absorbed.

The dose necessary to influence the serum iron level in normal subjects by peroral administration of iron is about 50 to 100 mg. The height of the serum iron increase is roughly directly proportional to the quantity given up to a point at which intestinal irritation is great enough to interfere materially with intestinal motility (137). This seems to coincide with the point where the total iron-binding capacity of plasma is fully utilized and the disturbed intestinal motility which is manifested by diarrhoea may depend on a local irritation of the intestinal wall by the increased Fe ion activity obtained when the total iron-binding capacity of plasma is exceeded. The serum iron absorption curves are often higher following the ingestion of ferrous than ferric salts (88, 137, 139, 140).

The literature on serum iron curves after Fe-loadings in patients with different diseases has been comprehensively reviewed by Kooyman (113), Waldenström (207), Jasinski (103, 104) and Roth (164). The results of the different investigators are not quite comparable because varying doses of iron have been used. The average increase of the serum iron varies between 50 and 100 μg per cent after loading normal subjects with iron salts (82, 104, 132, 147, 164, 200). In patients with iron deficiency after chronic loss of blood (haemorrhages from the gastrointestinal tract or the uterus), the rise of the serum iron is about 200–250 μg per cent (79, 82, 105, 106, 132).

The high serum iron increase in these patients is followed by a rapid decrease probably depending on an excessive elimination rate, as also seen after intravenous administration. The same type of curve has been observed in children with iron defiency (107, 164) and in some cases of polycythaemia vera (151, 207). Patients with other iron deficiency states, such as essential (idiopathic) hypochromic anaemia, chlorosis etc., do not regularly react to iron ingestion with a supranormal serum iron rise, but a normal or subnormal rise may be obtained (79, 82, 102). Typical iron deficiency curves may, however, be obtained if the iron loading is repeated after some weeks of iron therapy (190).

During acute infections not only is the serum iron lowered, but the resorption curve is also depressed. In some cases the serum iron values may even be unchanged after the loading (34, 104, 173). The curves in subjects with chronic infections (34, 113, 147) and malignant tumours (105, 207) are similar to those in patients with acute infections, but the abnormality is less pronounced.

Subnormal serum iron increase is also obtained in untreated patients with pernicious anaemia (207), haemochromatosis (18) and other diseases with hyperferraemia. In such cases the probable explanation is that the transferrin is already almost or completely saturated with iron before the loading. A normal rise has been observed (190, 207) in acute hepatitis in spite of the hyperferraemia (corresponding to the increased transferrin concentration in the blood of such patients). However, most cases of acute hepatitis show a subnormal serum iron increase after the iron ingestion (17).

After gastrectomy a subnormal serum iron increase may be obtained, probably on account of impaired iron absorption (123).

Influence of vitamins, hormones and the central nervous system on the serum iron level

There is no connection between vitamin C and the intermediate iron metabolism (198). Peroral or parenteral administration of aneurin, nicotinic acid amide, pyridoxin, and pantothenic acid has no influence on the serum iron level in normal subjects (118, 145, 146), but the serum iron value decreases slightly after intravenous injection of large amounts of lactoflavin (145, 186). Pyridoxin deficiency in dogs is followed by an inhibited bone marrow activity and hyperferraemia (75, 134, 217). Folic acid and B_{12} as well as liver extracts supplied to patients with pernicious anaemia are followed by hypoferraemia during the period of blood regeneration.

Whereas the serum iron level seems to be uninfluenced by nicotinic acid amide, hyperferraemia and slight hyperbilirubinaemia are regularly produced in normal subjects 90 minutes after peroral or intravenous administration of 20–50 mg nicotinic acid (17, 122). This effect seems to depend on an increased catabolism of the erythrocytes. The transferrin level remains unchanged (122).

The serum iron values fall within the normal variation range in patients suffering from most endocrine disorders (85, 113, 128). A slight hyperferraemia has relatively regularly been found in patients with pituitary hypofunction and after experimental hypophysectomy (47). In normal subjects the serum iron level is unchanged after injection of ACTH (39), testosterone, stilboestrol and desoxycorticosterone (128). In rabbits of both sexes testosterone and progesterone are without effect, but injections of oesterone preparations result in a rapid decrease of the plasma iron level (152). The presence of a hypothalamic centre for serum iron regulation has been proposed (62, 84, 85, 177, 190) This hypothesis is based on the existence of the diurnal serum iron variation and the fact that a slight serum iron decrease often comes about some hours after lumbar puncture, encephalography or electroshock (22, 85, 99, 177). Such treatment of the patient may, however, cause a stress and it is furthermore known that a reticulocytosis may appear after lumbar puncture (81). The observed serum iron variations may also be explained as secondary to changes in the functional state of the bone marrow or the reticulo-endothelial system.

Serum iron and the functional state of the reticulo-endothelial system

Dogs in which sterile turpentine abscesses were produced developed within 48 hours a pronounced hypoferraemia and a decreased transferrin level (35), followed by a slight anaemia (162). These changes are similar to those found during infection (80), and after injections of toxins (196) or species-foreign proteins (80, 205). This hypoferraemia is combined with accumulation of iron in the reticulo-endothelial system and with an increased elimination rate of iron from plasma (34, 147). It has therefore been assumed that the reticulo-endothelial system is of importance for the regulation of the serum iron level (78). Experiments to block the reticulo-endothelial system have given results which are difficult to interpret. Injection of electrocollargol (177) or colloidal thorium dioxide (38, 196) results within 24 hours in hypoferraemia in animals (rabbit, dog). On the other hand, if the administration of thorium dioxide to dogs is continued for 3 consecutive days, a considerable increase of the plasma iron (20 to 1500 per cent) is developed following the initial decrease (38). This increase generally begins on the first day following the last injection and reaches a maximum on the 3rd to the 7th day. This hyperferraemia is combined with an increase of the total iron-binding capacity of serum to more than double the initial values. Such hyperferraemic animals do not react with hypoferraemia and decreased total iron-binding capacity upon injection of turpentine (38).

An acute hypoferraemia (within 3–8 hours) can be produced in dogs by injections of large amounts of histamine (40, 174, 175), epinephrine (40, 42, 177), adrenal cortical extracts and ACTH (40). Administration of dibenamine to the animals before the injection of epinephrine does not prevent the hypoferraemia (40). The effect of these drugs on the transferrin level has not been reported.

That the effect of ACTH is mediated through the adrenal cortex is demonstrated by the fact that no hypoferraemia is produced by the injection of ACTH in adrenalectomized dogs (40). The hypoferraemic effect of epinephrine is significantly less in adrenalectomized dogs than in intact animals. Additional evidence of a connection between the adrenal cortex and the functional state of the reticulo-endothelial system is afforded by the experiments on the rate of disappearance from the plasma of intravenously injected saccharated oxide of iron. As mentioned, the iron given in this form is taken up by the reticuloendothelial system during the first phase of metabolism (9). The colloidal iron is removed more rapidly from the plasma of intact dogs given ACTH, and more slowly from the plasma of adrenalectomized dogs and from dogs previously given thorium dioxide (39, 40).

Serum iron turnover

In man, the amount of iron required daily for haemoglobin synthesis, calculated from the mean life time of the red blood cells, is about 20 to 25 mg. If the main part of the aged red blood cells is destroyed outside the bone marrow, this implies that an amount of iron about six times greater than the total serum iron quantity of about 4 mg. is daily transported to and consumed by the bone marrow. The actual serum iron turnover has been determined in human subjects after injection of tracer quantities of highly radioactive iron (95) and of transferrin labeled with radioactive iron (214). The disappearance of the radioactive iron from the circulating plasma was exponential and the half-time of the disappearance in the normals was about 100 minutes (95, 214). From this figure the average value for plasma iron turnover was calculated to be 27 mg. per day (95). From the amount of radioactive iron found in the haemoglobin of the red blood cells a certain time after the administration the average value for the fraction of red blood cell iron renewed per day was estimated to be 0.85 per cent, which corresponds to about 75 per cent of the calculated total plasma iron turnover. If the iron stores were filled up the rate of removal of radioactive iron from the serum was found higher than normal in patients with excessive bone marrow activity (*e.g.*, polycythaemia vera, haemolytic anaemia, untreated pernicious anaemia and occasionally in leukaemias), and lower than normal in patients with subnormal bone marrow activity (*e.g.*, aplastic anaemia and uraemia) (214). This suggests strongly that the rate of removal of radioactive iron is a true index of the production of red blood cells in the body (214). The influence of the iron stores on the disappearance rate of injected radioactive iron is small in comparison with the influence of the bone marrow (214). Thus, in a patient with polycythaemia, Wasserman found that the half-life time of radioactive iron in plasma increased from 30 to 145 minutes after treatment with radioactive phosphate (214). Feeding experiments performed with normal foodstuffs labeled with radio iron and fed to normal subjects indicated that probably less than one per cent of the total plasma iron turnover was concerned with absorption (95).

Previously Cruz *et al.* showed that the newly liberated iron from destroyed red blood cells in normal dogs is very effectively utilized in blood regeneration even though ample storage iron is available (49). This means that the exchange between the plasma iron and the storage iron is slower than between the plasma and the iron newly liberated from catabolized red blood cells. The relatively slow exchange between plasma iron and storage iron is also evident from the already mentioned fact that the increased serum iron level following an injection of some milligrams of iron is only slowly normalized in normal subjects in spite of the rapid iron turnover. The hypoferraemia which remains for weeks following an acute haemorrhage in subjects with normal iron stores may also be explained by a relatively slow exchange of iron between the plasma and the stores, *i.e.*, a slow mobilization of iron from the depots.

In this way all experimental evidence indicates that the main part of the red blood cells is normally destroyed outside the bone marrow and that the rate of erythropoiesis is the principal factor determining the serum iron turnover.

In consequence, the relatively stable serum iron level in normal subjects must mean that the formation and destruction of the red blood cells are going on during the whole day at approximately the same relative rate. It is reasonable to assume that the diurnal serum iron variation depends on a somewhat lower rate of incorporation of iron in the immature erythrocytes during the night when compared with the rate of liberation of iron from destroyed cells.

The turnover of transferrin has not been determined but it is reasonable to assume that it is of the same magnitude as that of other serum proteins.

DISCUSSION

The data thus far collected concerning the metabolism of transferrin and serum iron support the theory that the transferrin in plasma is the carrier of iron and that the iron leaves and enters the blood stream in ionized form (121). The mechanism regulating the transferrin concentration is a little known as the mechanism regulating the concentration of other serum proteins. We only know that the transferrin concentration increases during enhanced requirement for iron and that it decreases during haemolytic and pernicious anaemia, during infections and haematologically allied conditions (see page 381). The diseases which are followed by a decreased transferrin level are on the whole those in which there is a decreased level of serum albumin. The regular increase of the transferrin concentration of the blood during iron deficiency and pregnancy strongly supports the assumption that the transferrin level is of importance for iron mobilization and absorption.

The only likely explanation of the proposed influence of transferrin on iron metabolism is based on the following reversible reaction: Fe^{+++} + Fe-free transferrin $\rightleftarrows$ Fe-transferrin. The corresponding equilibrium is expressed by the equation: $\frac{[\text{Fe-transferrin}]}{[\text{Fe-free transferrin}]} = K \cdot [Fe^{+++}]$. The influence of the transferrin level on the concentration of ionized iron in plasma can be comprehended from this formula and further it is clear that the plasma in this way may mediate an equilibrium between the iron ion activity in the different organs of the body. This hypothesis explains why iron is mobilized from the iron stores during increased iron demand in the bone marrow and during pregnancy when the iron is lost to the fetus. Under these conditions the iron ion activity decreases in plasma and a concentration fall is established between the body cells and the plasma.

The hypothesis is purely schematic and factors such as pH and "redox" levels in plasma and the different cells are certainly of great importance for the actual iron ion activity. We do not know whether only ferrous iron or both ferrous and ferric iron can pass the cell membranes. The solubility of ferrous iron is much higher in the physiological range of pH than that of ferric iron, but even if the ferric iron concentration in plasma is extremely small this does not speak against the hypothesis since the equilibrium reaction between iron and transferrin is very rapid. We know that blood regeneration is increased during the weeks following an acute haemorrhage and during polycythaemia in spite of hypoferraemia. The average half-life time of plasma iron in patients with markedly increased serum iron turnover (*e.g.*, in polycythaemia vera) can be computed to be about 10 to 30 minutes from the figures given by Huff *et al.* (95); even in this case the main part of the administered iron is assimilated by the bone marrow, which is evident from the amount of radioactive iron appearing in the red blood cells. These results must signify that during increased haemopoietic activity more than 10 per cent of the total amount of the plasma iron passing through the bone marrow is retained even when the calculation includes a rather high minute volume for the bone marrow. This forms a biological proof for the rapid dissociation of the iron-transferrin complex in the body since no facts have been present which favour the theoretical possibility that the whole iron-transferrin complex is consumed in the bone marrow. The definite proof or disproof of the latter possibility can only be obtained by measuring the transferrin and the plasma iron concentration in the arterial and the venous blood from red bone marrow.

The equilibrium between the iron ion activity in plasma and different organs is probably established rapidly. The variations of the serum iron can be explained by the assumption of a relatively slow exchange between ionized iron and the storage iron (ferritin). This is further supported by Granick's finding (71) that the maximal ferritin concentration in the intestinal mucosal cells after peroral iron loading is obtained after about 7 hours, but the highest iron ion activity is probably reached during the two first hours after the loading as seen, for example, from the early maximum of the serum iron values after peroral intake of iron.

All these points of view can explain the observed variations of the serum iron during health and disease as being caused by a series of factors of which the variations of the transferrin level, the amount of available storage iron, the relative rate between the formation and the destruction of the various iron containing porphyrine compounds, and the iron demand of the reticulo-endothelial system are the most important. The low serum iron during infections and allied conditions can be understood if it is assumed that the iron temporarily is accumulated in the reticulo-endothelial system in a form nonavailable for intermediate iron metabolism.

REFERENCES

1. Agner, K.: Crystalline horse liver catalase. Ark. Kemi, Min. & Geol., **16A:** 1-21, 1943.
2. Agner, K.: Kliniska laborationsmetoder. "Astra", Södertälje, Sweden, 573-576, 1947.
3. Agner, K., Andersson, N. and Nordenson, N.: Intravenous iron therapy. Acta haemat., 193-211, 1948.
4. Albers, H.: Eisen bei Mutter und Kind. Thieme, Leipzig 1941.
5. Albers, H.: Die mangelhafte Eisenversorgung des künstlich ernährten Säuglings. Arch. Gynäk. Kongressband, **173:** 324-332, 1942.
6. Alderton, G., Ward, W. and Fevold, H.: Identification of the bacteria-inhibiting, iron-binding protein of egg white as conalbumin. Arch. Biochem., **11:** 9-13, 1946.

7. Althausen, T., Doro, R., Weiden, S., Motteram, R., Turner, C. and Moore, A.: Hemochromatosis. Arch. Int. Med., **88**: 553-570, 1951.

8. Amato, M.: Behaviour of serum iron in various cases of Cooley's anemia of children. Riv. clin. pediat., **44**: 711-714, 1946.

9. Andersson, N.: Experimental and clinical investigations into the effect of parenterally administered iron. Acta med. Scandinav. Suppl., **241**: 1-71, 1940.

10. Aussannaire, M. and Lafontaine, A.: Deux tests du diagnostic de l'hémochromatose: la ponction-biopsie du foie et le dosage du fer et du cuivre seriques. Rev. du foie, **2**: 158, 1946.

11. Barkan, G.: Eisenstudien. Die Verteilung des leicht abspaltbaren Eisens zwischen Blutkörperchen und Plasma und sein Verhalten unter experimentellen Bedingungen. Ztschr. f. physiol. Chem., **171**: 194-222, 1927.

12. Barkan, G.: Über Bestimmungsmethodik und Eigenschaften des "leicht abspaltbaren" Bluteisens. Ztschr. f. physiol. Chem., **216**: 1-17, 1933.

13. Barkan, G.: Die Unterscheidung des "leicht abspaltbaren" Bluteisens vom Hämoglobineisen und vom anorganischen Eisen. Ztschr. f. physiol. Chem., **221**: 241-251, 1933.

14. Barkan, G. and Schales, O.: Chemischer Aufbau und physiologische Bedeutung des "leicht abspaltbaren" Bluteisens. Ztschr. f. physiol. Chem., **248**: 96-116, 1937.

15. Barkan, G. and Walker, B.: The red blood cell as a source of the iron and bilirubin of the blood plasma. J. Biol. Chem., **131**: 447-454, 1939.

16. Barkan, G. and Walker, B.: Determination of serum iron and pseudohemoglobin iron with o-phenanthroline. J. Biol. Chem., **135**: 37-42, 1940.

17. Belsasso, E. and Zucconi, V.: Richerche sul ricambio del ferro nelle epatopatie. Rev. Gastroenterol., **2**: 117-141, 1950.

18. Belsasso, E. and Zucconi, V.: Richerche sul ricambio del ferro negli epatopazienti. Rev. Gastroenter., **2**: 219-240, 1950.

19. Biörk, G.: Blood iron—influence of exercise on serum iron in man. Acta physiol. scandinav., **15**: 193-197, 1948.

20. Bjerre, S. and Christoffersen, N.: Serum iron determination in diagnosis of acute hepatitis and other forms of jaundice. Nord. med., **16**: 3314-3317, 1942.

21. Bjerre, S. and Christoffersen, N.: Serum iron as diagnostic aid in acute hepatitis. Nord. med., **23**: 1345-1347, 1944.

22. Brenner, W.: Iron and copper metabolism in childhood. Ztschr. f. Kinderh., **66**: 14-35, 1948.

23. Brenner, W.: Iron and copper metabolism in infancy III. Ztschr. f. Kinderh., **66**: 299-318, 1949.

24. Brown, E., Moore, C., Reynafarje, C. and Smith, D.: Intravenously administered saccharated iron oxide in the treatment of hypochromic anemia. Therapeutic results, potential dangers and indications. J. Am. M. A., **144**: 1084-1089, 1950.

25. Brøchner-Mortensen, K. and Olsen, C.: Colorimetric method for determination of iron in serum with investigation of iron content of normal human serum. Compt. rend. trav. lab. Carlsberg, **23**: 235-248, 1940.

26. Brøchner-Mortensen, K.: Iron content of the serum in lesions of the liver and bile passages. Acta med. Scandinav., **112**: 277-290, 1942.

27. Brøchner-Mortensen, K.: Iron content of the serum in patients with hemorrhagic anemia. Acta med. Scandinav., **113**: 345-361, 1943.

28. Brøchner-Mortensen, K.: Om serumjern. Bibliot. laeger, **135**: 501-550, 1943.

29. Burch, H., Lowry, O., Bessey, O. and Berson, B.: Blood iron: determination in small volumes of serum. J. Biol. Chem., **174**: 791-802, 1948.

30. Büchmann, P.: Die Bedeutung der Serumeisenbestimmung für die Klinik. Ergebn. inn. Med. u. Kinderh., **60**: 446-507, 1941.

31. Büchmann, P.: Eisenresorption und Klinik. Ergebn. inn. Med. u. Kinderh., **64**: 505-624, 1944.

32. Cameron, D., Bensley, E., Wood, P. and Graystone, V.: Treatment of iron deficiency anaemia with saccharated iron oxide given by the intravenous route. Canad. Med. A. J., **64**: 27-30, 1951.

33. Cameron, D., Bensley, E. and Wood, P.: Some effects of intravenous injections of saccharated iron oxide on serum iron and unsaturated iron binding capacity. Canad. M. A. J., **64**: 30-31, 1951.

34. Cartwright, G., Lauritsen, M., Jones, P., Merrill, I. and Wintrobe, M.: Anemia of infection I. Hypoferremia, hypercupremia and alterations in porphyrin metabolism in patients. J. Clin. Investigation, **25**: 65-80, 1946.

35. Cartwright, G., Lauritsen, M., Jones, P., Merrill, I. and Wintrobe, M.: Anemia of infection II. Experimental production of hypoferremia and anemia in dogs. J. Clin. Investigation, **25**: 81-86, 1946.

36. Cartwright, G., Hughley, C., Aschenbrucker, H., Fay, J. and Wintrobe, M.: Studies on free erythrocyte protophorphyrin, plasma iron and plasma copper in normal and anemic subjects. Blood, **3**: 501-525, 1948.

37. Cartwright, G., Black, P. and Wintrobe, M.: Studies on the iron-binding capacity of serum. J. Clin. Investigation, **28**: 86-98, 1949.

38. Cartwright, G., Gubler, C. and Wintrobe, M.: The anemia of infection. XII. The effect of turpentine and colloidal thorium dioxide on the plasma iron and plasma copper of dogs. J. Biol. Chem., **184**: 579-587, 1950.

39. Cartwright, G., Gubler, C., Hamilton, L., Fellows, M. and Wintrobe, M.: The role of the adrenal cortex in the regulation of the plasma iron level and the functional state of the reticulo-endothelial system. Proc. 2. Clin. ACTH Conf., **1**: 405-412, 1951.

40. Cartwright, G., Hamilton, L., Gubler, C., Fellows, N., Aschenbrucker, H. and Wintrobe, M.: The anemia of infection. XIII. Studies on experimentally produced acute hypoferremia in dogs and the relationship of the adrenal cortex to hypoferremia. J. Clin. Investigation, **30**: 161-173, 1951.

41. Chaloupka, M. and Leverton, F.: Serum iron and hemoglobin of 275 healthy women. Proc. Soc. Exper. Biol. & Med., **77:** 677–680, 1951.

42. Cicconardi, A. and Postiglione, F.: The variations of serum iron in rabbits, poisoned by benzene and treated with intravenous adrenaline by Ascoli's method. Boll. Soc. ital. biol. sper., **24:** 835–837, 1948.

43. Cohn, E.: Chemical, physiological, and immunological properties and clinical uses of blood derivates. Experientia, **3:** 125–136, 1947.

44. Cohn, E., Gurd, F., Surgenor, D., Barnes, B., Brown, R. *et al.*: A system for the separation of the components of human blood. Quantitative procedures for the separation of the protein components of human plasma. J. Am. Chem. Soc., **72:** 465–474, 1950.

45. Cohn, E., Surgenor, D. and Hunter, M.: The state in nature of proteins and protein enzymes of blood and liver. Enzymes and enzyme systems, their state in nature. Edsall, Harvard University Press, Cambridge, 107–143, 1951.

46. Conte-Marotta, R. and Amato, M.: Serum iron levels in children at various stages of growth. Boll. Soc. ital. biol. sper., **18:** 24–26, 1943.

47. Crafts, R. and Walker, B.: Effects of hypophysectomy on serum and storage iron in adult female rats. Endocrinology, **41:** 340–346, 1947.

48. Cremer, J. and Münkel, O.: Über das Verhalten von Serumeisenzum Gesamtcholesterin beim Scharlach. Ztschr. f. d. ges. exper. Med., **113:** 566, 1944.

49. Cruz, W., Hahn, P. and Bale, W.: Hemoglobin radioactive iron liberated by erythrocyte destruction (acetylphenylhydrazine) promptly reutilized to form hemoglobin. Am. J. Physiol., **135:** 595–599, 1942.

50. Dahl, S.: Studies on serum iron. I. Serum iron in healthy women during the latter part of pregnancy, parturition and puerperium. Monatschr. f. Geburtsh. u. Gynäk., **119:** 281–303 1945.

51. Dahl, S.: Serum iron in normal women. Birt. M. J., **1:** 731–733, 1948.

52. De Franciscis, P. and Morsico, F.: Modification of serum iron in rabbits poisoned with benzene. Boll. Soc. ital. biol. sper., **24:** 683–685, 1948.

53. Dejardin, J. and Lambrechts, A.: Une méthod simplifieé de dosage du fer plasmatique. Acta biol. belg., **3:** 208–209, 1943.

54. de Raadt, M.: The iron metabolism in pernicious anemia. Acta med. Scandinav., **110:** 376–389, 1942.

55. Dreyfus, J. and Schapira, G.: Le fer sérique. Sang, **20:** 374–390, 1949.

56. Dreyfus, J., Schapira, G. and Schapira, F.: Le métabolisme du fer au cours de la cirrhose bronzée. Fer sérique et épreuve d'hypersidérémie provoquée. Bull. Acad. nat. méd., **134:** 302–304, 1950.

57. Dubach, R., Callender, S. and Moore, C.: Iron transportation and metabolism; absorption of radioactive iron in patients with fever and with anemias of varied etiology. Blood, **3:** 526–540, 1948.

58. Dubs, P.: Das Serumeisen in der Differentialdiagnose des Icterus. Schweiz. med. Wchnschr., **82:** 73–76, 1952.

59. Endicott, K., Gillman, T., Brecher, G., Ness, A., Clarke, F. and Adamik, E.: A study of histochemical iron using tracer methods. J. Lab. & Clin. Med., **34:** 414–421, 1949.

60. Fay, J. Cartwright, G. and Wintrobe, M.: Free erythrocyte protoporphyrin, serum iron, serum iron-binding capacity, and plasma copper during normal pregnancy. J. Clin. Investigation, **28:** 487–491, 1949.

61. Ferroni, A. and Indovina, I.: Oscillazioni giornaliere della sideremia nel parkinsonismo da encefalite. Acta neurol. (Naples), **2:** 167–174, 1947.

62. Ferroni, A. and Indovina, I.: Variazioni sideremiche dopo elettroshock ed elettroassenza. Acta neurol. (Naples), **2:** 898–903, 1947.

63. Fiala, S.: A study on the iron binding protein of plasma. Czechoslovak. Chem. Com. trav. chim. Tchécoslovaquie, **14:** 287–303, 1949.

64. Fiala, S. and Burk, D.: On the mode of iron by siderophilin, conalbumin hydroxylamine, aspergillic acid, and other hydroxamic acids. Arch. Biochem., **20:** 172–175, 1949.

65. Fontès, G. and Thivolle, L.: Sur la teneur du sérum en fer non hémoglobinique et sur sa diminution au cours de l'anémie expérimentale. Compt. rend. Soc. biol., **93:** 687–689, 1925.

66. Fraenkel-Conrat, H.: Comparison of the iron-binding activities of conalbumin and of hydroxylamidoproteins. Arch. Biochem., **28:** 452–463, 1950.

67. Fraenkel-Conrat, H. and Feeney, R.: The metal-binding activity of conalbumin. Arch. Biochem., **29:** 101–113, 1950.

68. Frandsen, S.: Iron metabolism in hemochromatosis. Acta med. Scandinav., **128:** 186–201, 1947.

69. Fuhrmann, G. and Barkemeyer, H.: A method for serum iron determination. Ztschr. ges. inn. Med., **2:** 458–460, 1947.

70. Goetsch, A., Moore, C. and Minnich, V.: The effect of massive doses of iron given intravenously to patients with hypochromic anemia. Blood, **1:** 129–141, 1946.

71. Granick, S.: Ferritin. IX. Increase of the protein apoferritin in the gastro-intestinal mucosa as a direct response to iron feeding. The function of ferritin in the regulation of iron absorption. J. Biol. Chem., **164:** 737–746, 1946.

72. Granick, S.: Structure and physiological functions of ferritin. Physiol. Rev., **31:** 489–511, 1951.

73. Greenberg, G., Aschenbrucker, H., Lauritsen, M., Worth, W., Humphreys, S. and Wintrobe, M.: Anemia of infection. V. Fate of injected radioactive iron in presence of inflammation. J. Clin. Investigation, **26:** 121–125, 1947.

74. Grinstein, M. and Moore, C.: Studies in iron transportation and metabolism. VII. Evidence obtained with radioactive iron that "easily split off" blood iron is an artefact. J. Clin. Investigation, **28:** 505–515, 1949.

75. Gubler, C., Cartwright, G. and Wintrobe, M.: The effect of pyridoxine deficiency on the absorption of iron by the rat. J. Biol. Chem., **178:** 989–996. 1949.

76. Hamilton, L., Gubler, C., Cartwright, G. and Wintrobe, M.: Diurnal variation in the plasma iron level of man. Proc. Soc. Exper. Biol. & Med., **75:** 65–68, 1950.

77. Heath, C., Strauss, M. and Castle, W.: Quantitative aspects of iron deficiency in hypochromic anemia; parenteral administration of iron. J. Clin. Investigation, **11:** 1293–1312, 1932.

78. Heilmeyer, L. and Ploetner, K.: Das Serumeisen und die Eisenmangelkrankheit. Fischer, Jena, 1937, 1–92.

79. Heilmeier L. and Koch H.: Absorption of iron under normal and pathological conditions. Deutsches. Arch. f. klin. Med., **185:** 89–101, 1940.

80. Heilmeyer, L., Keiderling, W. and Stüwe, G.: Kupfer und Eisen als körpereigene Wirkstoffe. Fischer, Jena, 1941, 1–132.

81. Heilmeyer, L.: Blutkrankheiten. Hdg. inn. Med. Springer, Berlin, 1942.

82. Hein, M. and Kleibl, E.: Klinische Beobachtungen über die Wirksamkeit von Ferronicum. Klin. Med., **5:** 244–252. 1950.

83. Hemmeler, G.: Serum Fe levels in parenchymatous and obstructive jaundice. Schweiz. med. Wchnschr., **73:** 1056–1060, 1943.

84. Hemmeler, G.: Matabolism of Fe; diurnal fluctations in serum Fe. Helvet. med. acta, **11:** 201–207, 1944.

85. Hemmeler, G.: Métabolisme du fer. Physiologie-pathologie traitement. Masson & Cie, Bd St. Germain, Paris, VI^{e}, 1951, 1–257.

86. Henriques, V. and Roche, A.: Sur le fer du sérum sanguin de diverses espèces animales. Bull. Soc. chim. biol., **9:** 501–504, 1927.

87. Hirvonen, M.: The iron content of serum in some ordinary infections. Acta. med. Scanidnav., **106:** 495–515, 1941.

88. Hoet, J. and Lederer, J.: Les bases physiologiques de la thérapeutique martiale. Bull. de Galenica, Genève, 1947, 1–72.

89. Holly, R.: Iron metabolism in pregnancy. Bull. of the Univ. of Minnesota Hosp. and Minnesota Med. Foundation, **20:** 475–500, 1949.

90. Holly, R.: Intravenous iron: Evaluation of the use of saccharated iron oxide in iron deficiency states in obstetrics and gynecology. Blood, **6:** 1159–1172, 1951.

91. Holmberg, C. G. and Laurell, C.-B.: Studies on the capacity of serum to bind iron. The regulation mechanism of serum iron. Acta physiol. Scandinav., **10:** 307–319, 1945.

92. Holmberg, C. G. and Laurell, C.-B.: Investigations in serum copper. I. Nature of serum copper and its relation to the iron-binding protein in human serum. Actajcham. Scandinav., **1:** 944–950, 1947.

93. Holmberg, C. G. and Laurell, C.-B.: Investigations in serum copper. II. Isolation of the copper containing protein, and a description of some of its properties. Acta chem. Scandinav., **2:** 550–556, 1948.

94. Horrigan, D., Mueller, J. and Vilter, R.: Observations of the intravenous administration of saccharated oxide of iron in human beings. J. Lab. & Clin. Med., **36:** 422–427, 1950.

95. Huff, R., Hennessy, T., Austin, R., Garcia, J., Roberts, M. and Lawrence, J.: Plasma and red cell iron turnover in normal subjects and in patients having various hematopoietic disorders. J. Clin. Investigation, **29:** 1041–1052, 1950.

96. Høyer, G.: Absorption-photometric determination of serum iron with 1,10-phenanthroline. Scandinav. J. Clin. & Lab. Investigation, **2:** 125–132, 1950.

97. Høyer, K.: Blood iron—physiologic variation in content of human serum; variations from week to week, from day to day and through 24 hours. Acta med. Scandinav., **119:** 562–576, 1944.

98. Høyer, K.: Blood iron—physiologic variations in content of human serum; further studies of intra diem variations. Acta med. Scandinav., **119:** 577–585, 1944.

99. Indovina, J.: Fisiopatologia del ferro. Agnini, Catania, 1948.

100. Iversen, P. and Warming-Larsen, A.: Case of hemochromatosis diagnosed by liver biopsy. Acta med. Scandinav. Suppl., **206:** 344–349, 1946.

101. Jager, B.: Immunological studies of an iron-binding protein in human serum. J. Clin. Investigation, **4:** 792, 1949.

102. Jasiński, B.: Eisenmangelzustände und ihre Therapie mit neuen Ferrosalzen. Helvet. med. acta, **16:** 67–89, 1949.

103. Jasiński, B.: Zur Frage der nicht- und der leichtanämischen Eisenmangelkrankheit. Praxis, **38:** 811–816, 1950.

104. Jasiński, B.: Resorptionstypen nach peroraler Eisenbelastung mit Ferronicum. Schweiz. med. Wchnschr., **80:** 59–62, 1950.

105. Jasiński, B.: Eisenresorption und Infektion. Erkennung des Eisenmangels bei Infektionskrankheiten durch Belastungsversuch. Pathogenese der Infektanämie. Acta haemat., **3:** 17–26, 1950.

106. Jasiński, B. and Diener, E.: Zur Pathogenese der Anämie bei Myomen. Gynaecologia, **130:** 233–240, 1950.

107. Jasiński, B.: Ist die leichte Anämie des Kindes ein physiologischer Zustand? Ann. paediat., **177:** 129–140, 1951.

108. Kartchner, F. and Holmstrom, E.: The treatment of iron deficiency anemia of pregnancy with intravenous iron. Am. J. Obst. & Gynec., **60:** 1288–1297, 1950.

109. Keiderling, W.: Über den Kupfer-Eisen-Antagonismus in Blutplasma und seine klinische Bedeutung als unspezifische humorale Abwehrreaktion bei tuberkulösen Krankheitsprozessen. Beitr. z. Klin. Tuberk., **103:** 447–458, 1950.

110. Kitzes, G., Elvehjem, C. and Schuette, H.: Determination of blood plasma iron. J. Biol. Chem., **155:** 653–660, 1944.

111. Klopper, A. and Ventura, S.: Iron metabolism in pregnancy. Brit. M. J., **2:** 1251–1253, 1951.

112. Koechlin, B.: Preparation and properties of serum and plasma proteins. Crystallization and characterization of a metal-combining β_1-psuedoglobulin from human plasma. In press. J. Am. Chem. Soc. 1952.

113. Kooyman, J.: De Ijzerstofwisseling. G. W. Van der Wiel & Co., Arnhem, 1947, 1–148.

114. Kuhns, W., Cartwright, G. and Wintrobe, M. Anemia of infection: clinical experiences with ferrivenin. Am. J. Med., **8:** 523, 1950.

115. Kuhns, W., Gubler, C., Cartwright, G. and Wintrobe, M.: The anemia of infection. XIV. Response to massive doses of intravenously administered saccharated oxide of iron. J. Clin. Investigation, **29:** 1505–1513, 1950.

116. Lambrechts, A. and Plumier, M.: Recherches sur le "fer facilement libérable". Acta biol. belg., **2:** 235–238, 1942.

117. Lapicque, L.: Une vieille donée perdue de vue; le foie de la femme contient deux fois et demi moins de fer que celui de l'homme. Compt. rend. Soc. biol., **141:** 214, 1947.

118. Lattanzi, A.: Vitamine idrosolubili e ricambio del ferro. Arch. stud. fisiopat., **13:** 148–165, 1949.

119. Laurell, C.-B. and Ingelman, B.: The iron-binding protein of swine serum. Acta chem. Scandinav., **1:** 770–776, 1947.

120. Laurell, C.-B.: Studies on the transportation and metabolism of iron in the body. Acta physiol. Scandinav. Suppl., **46:** 1–129, 1947.

121. Laurell, C.-B.: What is the function of transferrin in plasma. Blood, **6:** 183–187, 1951.

122. Laurell, C.-B.: Unpublished observations.

123. Lederer, J.: L'anémie agastrique hypochrome ferriprive et sa pathogénie. Acta gastroenterol. belg., **14:** 445–452, 1946.

124. Lederer, J.: Les travaux belges sur la physio-pathologie du fer dans l'organism vivant. Arch. med. belg. Juillet, 241–259, 1946.

125. Lederer, J.: Sur la nature du fer sérique. I. Étude de la dialyse du fer sérique et du fer ajouté au sérum. Arch. internat. de pharmacodyn. et de thérap., **76:** 383–396, 1948.

126. Lederer, J.: Sur la nature du fer sérique. II. Étude de la fraction IV-7 des globulines. Arch. internat. de pharmacodyn. et de thérap., **77:** 104–110, 1948.

127. Lederer, J. and Renaer, M.: La thérapeutique martiale parentérale par le cacodylate ferric. J. Suisse de Médecine, **77:** 1061–1065, 1947.

128. Lemaire, A., Loeper, J. and Loeper, J.: Serum iron in various hepatic and endocrine disorders. Presse méd. **57:** 1117–1118, 1949.

129. Lemberg R. and Legge, J.: Hematin compounds and bile pigments. Interscience publishers, Inc., New York, 1949, 1–745.

130. London, I., Shemin, D. and Rittenberg, D.: Synthesis of heme in vitro by the immature nonnucleated mammalian erythrocyte. J. Biol. Chem., **183:** 749–755, 1950.

131. Lundström, P.: Studies on erythroid elements and serum iron in normal pregnancy. Acta Soc. med. Upsaliensis **55:** 1–84, 1949.

132. Lüdin, H.: Klinische Erfahrungen mit oraler Applikation von zwei organischen Eisenpräparaten. Schweiz. med. Wchnschr., **79:** 1–15, 1949.

133. Maier, C.: Hämolyse und hämolytische Krankheiten. Hans Huber Verlag, Bern. 1950, 1–316.

134. McKibbin, J., Schaefer, A., Frost, D. and Elvehjem, C.: Anemia in dogs due to pyridoxine deficiency. J. Biol. Chem., **142:** 77–84, 1942.

135. Moore, C., Arrowsmith, W., Quiligan, J. and Read, J.: Studies in iron transportation and metabolism. I. Chemical methods and normal values for plasma iron and "easily split-off" blood iron. J. Clin. Investigation, **16:** 613–626, 1937.

136. Moore, C., Doan, C. and Arrowsmith, W.: Studies in iron transportation and metabolism. II. The mechanism of iron transportation, its significance in iron utilization in anemic states of varied etiology. J. Clin. Investigation, **16:** 627–648, 1937.

137. Moore, C., Minnich, V. and Welch, J.: Studies in iron transportation and metabolism. III. The normal fluctuations of serum and "easily split-off" blood iron in individual subjects. J. Clin. Investigation, **18:** 543–552, 1939.

138. Moore, C., Arrowsmith, W., Welch, J. and Minnich, V.: Studies in iron transportation and metabolism. IV. Observations on the absorption of iron from the gastro-intestinal tract. J. Clin. Investigation, **18:** 553–580, 1939.

139. Moore, C., Discussion of iron metabolism. J. Pediat., **17:** 547–556, 1940.

140. Moore, C., Dubach, R., Minnich, V. and Roberts, H.: Absorption of ferrous and ferric radioactive iron by human subjects and by dogs. J. Clin. Investigation, **23:** 755–767, 1944.

141. Neander, G. and Vahlquist, B.: Studies on the absorption of iron. III. The serum iron curve in rabbits following intravenous administration of iron in massive doses. Acta physiol. Scandinav., **17:** 110–119, 1949.

142. Neukomm, S.: Contribution à l'étude des rapports entre le fer sérique et les proteines plasmatiques. Helvet. med. acta, **14:** 453–457, 1947.

143. Neukomm, S.: La régulation physico-chimique de la sidérémie. Acta haemat., **2:** 213–233, 1949.

144. Neuweiler, W.: Serum iron during pregnancy and the postpartum period. Ztschr. f. Geburtsh. u. Gynäk., **124:** 252–263, 1942.

145. Neuweiler, W.: The influence of water-soluble vitamins (C, B_1, B_2, nicotinic acid, and pantothenic acid) on the iron and copper levels in the serum. Ztschr. Vitaminforsch., **16:** 1–11, 1945.

146. Neuweiler, W.: Influence of pyridoxine and becozyme (vitamin B complex preparation) on iron and copper levels of serum. Ztschr. Vitaminforsch., **18:** 70–74, 1946.

147. Nilsson, F.: Anaemia problems in rheumatoid arthritis. Acta med. Scandinav. Suppl., **210:** 1–193, 1948.

148. Nissim, J.: Iron toxicity, intravenous administration of iron. Lancet, **2:** 49–51, 1947.

149. Nissim, J. and Robson, J.: Preparation and standardisation of saccharated iron oxide for intravenous administration. Lancet, **1:** 686–689, 1949.

150. Olivia, G.: La sideremia e il ricambio del ferro in condizioni normali e patologiche. Luigi Pozzi, Roma 1948, 1–256.

151. Olivia, G. and Frascarelli, R.: Ricambio del ferro e ricambio emoglobinico nella policitemia vera. Sett. med. **36**: 170–174, 1948.

152. Palmer, H.: The influence of sex hormones on plasma iron. Scandinav. J. Clin. & Lab. Invest. **4**: 81, 1952.

153. Perosa, L.: Il metabolismo del Fe e la dissociazione ipocromiaipersideremia nelle sindromi emopatiche mediterranee. Riforma med., **34**: 807, 1949.

154. Perosa, L.: Il Fe eritrocitario nel M. di Cooley e nelle sindromi emopatiche affini. Prog. med., Napoli, **6**: 1–8, 1950.

155. Plumier, M. and Lambrechts, A.: L'étude du fer plasmatique dans l'ictère et l'anémie pérnicieuse. Acta biol belg., **4**: 393–395, 1941.

156. Powell, J.: Serum iron in health and disease. Quart. J. Med., **13**: 19–26, 1944.

157. Prinzie, A.: Étude de la dialyse du fer ajouté aux fractions des proteins plasmatiques. Arch. internat. de pharmacodyn. et de thérap., **86**: 267–291, 1951.

158. Rath, C. and Finch, C.: Measurement of iron binding capacity of serum in man. J. Clin. Investigation, **28**: 79–85, 1949.

159. Rath, C., Caton, W., Reid, D., Finch, C. and Conroy, L.: Hematologic changes and iron metabolism of normal pregnancy. Surg., Gynec. & Obst., **90**: 320–326, 1950.

160. Reginster, A.: Anemia and hyposideremia in tuberculosis. Schweiz. med. Wchnschr., **73**; 1295–1299, 1943.

161. Renaer, M. and Schockaert, J.: Étude comparée de la résorption du chlorure ferreux a la fin de la grossesse et au cours des premiers jours du post partum. Acta biol. belg., **2**: 36–39, 1942.

162. Robscheit-Robbins, F. and Whipple, G.: Infection and intoxication; their influence upon hemoglobin production in experimental anemia. J. Exper. Med., **63**: 767–787, 1936.

163. Roth, O. and Jasiński, B.: Hämolytische Anämien bei Panmyelopathie und ihre Pathogenese. Helvet. med. acta, **16**: 531–547, 1949.

164. Roth, O.: Über den Eisenstoffwechsel des Menschen. Mitteilungen der Naturwissenschaftlichen Gesellschaft in Winterthur, Heft, **26**: 3–23, 1951.

165. Roth, O., Jasiński, B. and v. Bidder, H.: Das Gewebeeisen beim Menschen bei normalen und pathologischen Zuständen. Helvet. med. acta, **18**: 59–174, 1951.

166. Rothlin, E. and Undritz, E.: Experimenteller Beitrag zur Kenntnis der larvierten ferripriven Anämie (Sideropenie ohne Anämie). Schweiz. med. Wchnschr., **77**: 58–66, 1947.

167. Rustung, E.: Serum iron. Acta dermat.-venereol. Suppl. **21**: 1–401, 1949.

168. Schade, A. and Caroline, L.: Raw hen egg white and the role of iron in growth inhbition of shigella dysenteriae, staphylococcus aureus, escherichia coli and saccharomyces cerevisiae. Science, **100**: 14–15, 1944.

169. Schade, A. and Caroline, L.: Iron-binding component in human blood plasma. Science, **104**: 340–341, 1946.

170. Schade, A., Reinhart, R. and Levy, H.: Carbon dioxide and oxygen in complex formation with iron and siderophilin, the ironbinding component of human plasma. Arch. Biochem., **20**: 170–172, 1949.

171. Schulman, I.: Proc. 2. ACTH Conf., **1**: 411, 1951.

172. Schaefer, K.: Zur Pathogenese der Infektanämie, insbesondere ihre Beziehungen zum Eisenstoffwechsel des wachsenden Organismus. Klin. Wchnschr., **19**: 590–594, 1940.

173. Schaefer, K.: Untersuchungen über den exogenen Eisenstoffwechsel bei fieberhaften Infekten im Kindesalter. Klin. Wchnschr., **19**: 979–982, 1940.

174. Schaefer, K.: Über den Einfluss von Infektionen und ähnlichen Vorgängen auf den Eisenstoffwechsel. Ztschr. f. d. ges. exper. Med., **110**: 678–731, 1942.

175. Schaefer, K.: Einfluss allergischer Vorgänge auf das Serumeisen. Monatschr. f. Kinderh., **96**: 18–23, 1948.

176. Schaefer, K.: Eisenstoffwechsel. Monatschr. f. Kinderh., **97**: 142–150, 1949.

177. Schaefer, K. and Boenecke, I.: Die neurovegetative Lenkung des Eisenstoffwechsels. Arch. f. exper. Path. u. Pharmakol., **207**: 666–687, 1949.

178. Scott, J. and Govan, A.: Anaemia of pregnancy treated with intravenous iron. Lancet, **1**: 367–370, 1951.

179. Segschneider, P.: Serumeisen bei der gesunden Frau und in der Schwangerschaft. Arch. f. Gynäk., **175**: 1–16, 1944.

180. Sisson, R., Smith, C., Bosnes, R., Siegal, S. and Stock, S.: Effect of metal-combining globulin in severe mediterranean anemia. Proc. Soc. Exper. Biol. & Med., **74**: 258–261, 1950.

181. Skjetne, R.: Serum iron and prothrombin in hepatic disease and jaundice, preliminary report. Nord. med., **38**: 733–736, 1948.

182. Skouge, E., Klinische und experimentelle Untersuchungen über das Serumeisen. Videnskabsadkademien (Oslo) Skrifter. Mat. Natv. Kl. Bd 1, No. 7: 1–173, 1939.

183. Sköld, E. and Waldenström, J.: Hemoglobin ach serumjänrvärden hos blodgivare. Svensk läkartidn., **37**: 1359–1367, 1940.

184. Smith, C., Sisson, T., Floyd Jr., W. and Siegal, S.: Serum iron and iron-binding capacity of serum in children with severe Mediterranean (Cooley's) anemia. Pediatrics, **5**: 799–807, 1950.

185. Starkenstein, S. and Harvalik, Z.: Über eine im intermediären Eisenstoffwechsel entstehende Ferriglobulinverbindung. Arch. f. exper. Path. u. Pharmakol., **172**: 75–92, 1933.

186. Stich, W. and Wolff, H.: Der Einfluss des Lactoflavin auf den Serumeisenspiegel. Klin. Wchnschr., **29**: 356, 1951.

187. Sundelin, G.: Om serumjärnet under gravidetet. Svensk läkartidn. **39**: 1451–1456, 1942.

188. Surgenor, D., Koechlin, B. and Strong, L.: Chemical, clinical, and immunological studies on the products of human plasma fractionation. J. Clin. Investigation, **28**: 73–78, 1949.

189. Surgenor, D., Strong, L., Taylor, H., Gordon Jr., R. and Gibson, D.: The separation of choline esterase mucoprotein, and metal-combining protein into subfractions of human plasma. J. Am. Chem. Soc., **71:** 1223-1229, 1949.

190. Thedering, F.: Die Bedeutung von Resorptionskurven nach Eisenbelastungen für die Beurteilung von Eisenmangelsanämien. Ztschr. ges. inn. Med., **3:** 547-558, 1948.

191. Thedering, F.: Zur Bindung des Transporteisens an die Plasmaproteine. Verhandl. deutsch. Gesellsch. inn. Med. 55. Kongress, Wiesbaden, 310-312, 1949.

192. Thedering, F.: Zur vegetativen Streuerung des Serumeisens. Klin. Wchnschr., **27:** 496-501, 1949.

193. Thedering, F.: Die Bindung des Serumeisens an die Plasmaproteine. Acta haemat., **3:** 210-229, 1950.

194. Thedering, F. and Gross, R.: Fortschritte mit der intravenösen Eisentherapie. Ztschr. ges. inn. Med., **4:** 634-641, 1949.

195. Thoenes, F. and Aschaffenburg, R.: Der Eisenstoffwechsel des wachsenden Organismus. Eine literatische, klinische und experimentelle Studie. Abhandl. Kinderheilk., **35:** 1-103, 1934.

196. Thoenes, F.: Physiologie und Pathologie des Eisenstoffwechsels im Wachstumsalter. Deutsche Ztschr. Verdauungskr., **4:** 209-234, 1941.

197. Tompsett, S. and McAllister, R.: Determination of the iron content of blood serum. Analyst, **74:** 315-316, 1949.

198. Tötterman, L.: Vitamin C and iron metabolism. Acta med. Scandinav. Suppl., **230:** 1-158, 1949.

199. Vahlquist, B.: Das Serumeisen, eine pädiatrisch-klinische und experimentelle Studie. Acta paediat. Suppl., **5:** 1-374, 1941.

200. Vahlquist, B.: Om järn och järnterapi. A. B. Ferrosan, Mamlö, 1944, 41-127.

201. Vahlquist, B.: The cause of the sexual differences in erythrocyte, hemoglobin and serum iron levels in human adults. Blood, **5:** 874-875, 1950.

202. Vannotti, A. and Delachaux, A.: Der Eisenstoffwechsel und seine klinische Bedeutung. Benno Schwabe, Basel, 1942, 1-212.

203. Vannotti, A. and Delachaux, A.: Iron metabolism. F. Muller, London, 1949, 1-267.

204. Ventura, S. and Klopper, A.: Iron metabolism in pregnancy. The behaviour of haemoglobin, serum iron, the iron-binding capacity of serum proteins, serum copper and free erythrocyte protoporphyrin in normal pregnancy. J. Obst. & Gynaec. Brit. Emp., **58:** 173-189, 1951.

205. Volland, W.: Untersuchungen über den intermediären Eisenstoffwechsel nach wiederholter Injektion artfremden Serums. Klin. Wchnschr., **19:** 1242, 1940.

206. Waldenström, J.: Determination of serum iron, practical significance. Nord. med., **11:** 2341-2346, 1941.

207. Waldenström, J.: Om järn och järnterapi. A. B. Ferrosan, Malmö, 1944, 55-110.

208. Waldenström, J.: Incidence of "iron deficiency" (sideropenia) in some rural and urban populations. Acta med. Scandinav. Suppl., **170:** 252-279, 1946.

209. Wallenius, G.: A note on serum iron transportation. Scandinav. J. Clin. & Lab. Investigation, **4:** 24-26, 1952

210. Walsh, J., Thomas, E., Chow, S., Fluharty, R. and Finch, C.: Iron metabolism—heme synthesis in vitro by immature erythrocytes. Science, **110:** 396-398, 1949.

211. Warburg, O.: Methode zur Bestimmung von Kupfer und Eisen und über den Kupfergehalt des Blutserums. Biochem. Ztschr., **187:** 255-271, 1927.

212. Warburg, O. and Krebs, H.: Überlocker gebundenes Kupfer und Eisen im Blutserum. Biochem. Ztschr., **190:** 143-149, 1927.

213. Warmer, R. and Weber, I.: The preparation of crystalline conalbumin. J. Biol. Chem., **191:** 173-180, 1950.

214. Wasserman, L., Rashkoff, I., Leavitt, D., Mayer, J. and Port, S.: The rate of radioactive iron from the plasma—an index of erythropoiesis. J. Clin. Investigation, **31:** 32-39, 1952.

215. Widdowson, E. and McCance, R.: Iron metabolism—sexual differences in storage and metabolism. Biochem. J., **42:** 577-581, 1948.

216. Widmer, W.: Beitrag zur fötalen Eisenversorgung. Schweiz. med. Wchnschr., **78:** 439-444, 1948.

217. Wintrobe, M., Greenberg, G. Humphreys, S., Aschenbrucker, H., Worth, W. and Kramer, R.: Anemia of infection: III. Uptake of radioactive iron in iron deficient and in pyridoxine-deficient pigs before and after acute inflammation. J. Clin. Investigation, **26:** 103-113, 1947.

218. Yoshikawa, H., Hahn, P. and Bale, W.: The form of combination of radio-active iron and copper in plasma following ingestion. Proc. Soc. Exper. Biol. & Med., **49:** 285-289, 1942.

THE PHARMACOLOGICAL ACTIONS OF MAGNESIUM IONS WITH PARTICULAR REFERENCE TO THE NEUROMUSCULAR AND THE CARDIOVASCULAR SYSTEM

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In reviewing an extensive field such as the pharmacology of magnesium ions it is necessary to limit the subject. The present review will deal mainly with the effects of an increase in magnesium concentration on the neuromuscular and cardiovascular system. Work on the effects of magnesium deficiency, the metabolism and therapeutic effect of magnesium ions will not be considered. Magnesium metabolism and the effects of magnesium deprivation have been reviewed by Schmidt and Greenberg (116) and Greenberg (58). The therapeutic effects were dealt with by Smith, Winkler and Hoff (123), who reviewed the pharmacological effects and the absorption and excretion of magnesium salts. Variations in serum magnesium concentration in various disease conditions were covered by Haury (67).

Central versus Peripheral Action of Magnesium in the Nervous System. The depressant action of magnesium on the neuromuscular system was first demonstrated by Jolyet and Cahours (75) who found a peripheral curare-like action in both cold- and warm-blooded animals. Meltzer and Auer (96), in an extensive series of papers on the anesthetic effect of magnesium salts, confirmed the depressant action but attributed it solely to a central anesthetic effect, a point of view they later revised by further attributing to magnesium a peripheral depressant action on neuromuscular transmission. At about the same time it was shown beyond doubt that magnesium in man has a depressant effect on the cortex, since unconsciousness is produced by injection of magnesium salts (110).

The problem of the site of action of magnesium, especially the effect on different parts of the central nervous system and the sensitivity of the central nervous system as compared with that of the neuromuscular junction, was the object of numerous discussions in the following years.

The first systematic investigations were made by Matthews and Brooks (94) using cross-circulation experiments in dogs. They found a central depressant action on respiration, though this was not as easily produced as the peripheral paralysing effect. More recent investigations of the action on the spinal reflexes show some discrepancies. The experiments of Hoff, Smith and Winkler (73) on decerebrate cats demonstrated that magnesium chiefly affects neuromuscular transmission and not the central nervous system, although the experiments do not exclude a central effect. They correlated the motor response produced by electrical stimulation of the motor nerve at different frequencies with the serum magnesium concentration. At high concentrations of serum magnesium, high frequencies of stimulation were required to produce a motor response. The different reflexes disappeared at different concentrations of serum magnesium: tendon reflexes at about 5 mM magnesium; respiration at approximately 10 mM;

whereas the corneal reflex did not disappear until a concentration of 15–18 mM of magnesium was reached. The order of disappearance of reflexes was correlated with the frequency of spontaneous reflex discharge characteristic of the motor element of the reflex, the reflexes with slow discharge frequency disappearing first. After tetanic stimulation of the afferent nerve, the reflexes could be made to reappear, suggesting to the authors that the site of effect on the reflexes was at the neuromuscular junction. On the other hand, Bryant *et al.* (22a), using a different technique, concluded that the action of magnesium is primarily central. They compared reflex activity and motor response to stimulation of the motor nerve during magnesium anesthesia, and found that spinal reflexes were abolished while neuromuscular transmission was still unimpaired. More recently a similar technique has yielded different results. The site of the depressant action of magnesium was investigated by recording the threshold for the crossed extensor reflex and the threshold for the response to electrical stimulation of the motor nerve. At the time of respiratory paralysis produced by magnesium salts, a reduced peripheral but unchanged central irritability was demonstrated. By further increasing the degree of anesthesia (during artificial respiration) a depression of the reflex centre occurred very soon (42). Hoff *et al.* (73) suggested that the discrepant findings of Bryant *et al.* (22a) might be due to the use of different frequency of stimulation. However, a high stimulation frequency can hardly be the explanation since Engbæk (42) with 100 c.p.s. still found primarily a peripheral action of magnesium.

In summarizing we may conclude that both central and peripheral depressant actions of magnesium have been demonstrated. The relative sensitivity seems to be greatest in the periphery of the motor system although this question is not finally settled.

Central Nervous System Effect of Magnesium. As mentioned above the intravenous injection of magnesium produces unconsciousness. The effect of magnesium on spontaneous electrical activity of the brain was first examined on the olfactory bulb of the isolated frog brain (85). Magnesium in concentrations of the same order of magnitude as the anesthetic concentrations in warmblooded animals (3–10 mM added to the magnesium-free Ringer's solution) produced slowing of the normal 6 per sec. rhythm. The electrocorticogram of cats under light pentobarbital anesthesia was likewise modified by intravenous infusion of magnesium, which at first produced a transient slowing (115), the effect being rather small as compared with the effect of aliphatic anesthetics. The slowing of the electrical activity reappeared later, simultaneously with electrocardiographic changes similar to those produced by calcium and potassium. But calcium and potassium did not alter the electrocorticogram until the heart was affected. As pointed out by Rubin *et al.* (115), the early transient changes in the electrocorticogram might be secondary to the fall in blood pressure simultaneously produced. However, before the heart is affected, the mean arterial blood pressure does not fall below 100 mm Hg (72), a fall in blood pressure which according to Beecher *et al.* (7) is not likely to produce changes in the electrocorticogram. A slowing in the electroencephalographic rhythm of the unanesthetized rabbit was also found by Bertrand *et al.* (9), but these experiments were performed without artificial respiration, and the effect might be due to the anoxemia produced by respiratory failure.

Interference by the cardiovascular effects of magnesium makes it difficult to demonstrate the effect of the ion on the spontaneous electrical activity of the brain in the intact animal. If it exists at all, it is rather small as compared with the effect of the aliphatic anesthetics.

Nerve Fibers. Block of conduction in peripheral nerve occurs at concentrations which are 10–20 times those needed for blocking neuromuscular transmission (138, 46, 29, 42). At this high concentration it is doubtful if the blocking is due to a specific effect of magnesium ions. To obtain this concentration either all the sodium of the Ringer's solution must be replaced by magnesium, which suggests that the lack of sodium in itself establishes conduction block (71), or the magnesium must be applied in a hypertonic solution. Moreover, the action potential of single nerve fibers of the frog is unaffected by concentrations up to 66 mM magnesium (68) (the concentration of a magnesium solution isotonic with frog Ringer's solution being approximately 80 mM). The effect of magnesium (20 mM $MgCl_2$) upon the membrane potential of the nerve consists in a slight initial increase, followed by slight depolarization after 2 to 3 hours (87). Even after 24 hours this depolarization amounted to only a few millivolts (about 10 per cent) and the nerve fibers could still conduct impulses without detectable abnormality. Excess of calcium exerted a similar effect, but its action was more rapid and less reversible than that of magnesium. The depolarization produced by anoxia was delayed by magnesium, the magnesium treated part of the anoxic nerve staying excitable for some time (87). A similarity in effects of magnesium and calcium on peripheral nerve was found in the effect on the temperature sensitivity of the spike potential, in that both magnesium and calcium lower the optimum temperature for maximum spike height (83).

It seems well established that the depressant action of magnesium on motor nerves is much less pronounced than its effect on neuromuscular transmission and on the central nervous system.

A certain local anesthetic effect has been claimed by several authors (96, 139, 63). As for the motor nerves, however, the experiments were made with hypertonic solutions or with sodium-free isotonic solutions, and the specific blocking effect on sensory nerves is doubtful.

Neuromuscular Transmission and Muscle. The primary peripheral effect of magnesium is its blocking effect on neuromuscular transmission. When a nerve-muscle preparation is treated with magnesium (20–50 mM) it becomes inexcitable to stimulation through the nerve, while the irritability of the muscle remains unchanged (75, 77, 5, 29). After prolonged applications, changes in the irritability of the muscle occur (10, 63, 138, 5, 36, 1). During magnesium anesthesia of the intact animal, the height of contraction of directly stimulated muscle was found to be unaffected, while stimulation of the motor nerve failed to produce a response. This was the case with a serum magnesium concentration up to 15 mM (73). The magnesium effect on the muscle has been examined on curarized and denervated muscle in order to avoid stimulation of the intramuscular nerve endings. In curarized frog muscle the height of contraction was reversibly reduced by 20 mM magnesium (138), 10 mM producing a transient small effect on contraction and a significant decrease in excitability (42). The threshold magnesium concentration for effect on the muscle is 10 mM in cold-blooded animals, while it must be higher for mammalian muscle (73).

In denervated frog muscle Ashkenaz (1) found a decrease in excitability, after application of 67 mM $MgCl_2$ in Ringer's solution, of the same order of magnitude as in normal muscle. The effect was, however, less reversible in the denervated than in the normal muscle.

Intravenous injection of magnesium decreased the twitch height of both the innervated and the denervated anterior tibialis muscle in the dog (90a). The depressant action was restored within a few minutes in the case of muscle with intact nerve supply, whereas the denervated muscle did not recover within half an hour. It seems advisable, however, not to transfer findings on denervated muscle directly to normal muscle. Several substances have a different mode of action on denervated muscle, possibly due to a difference in permeability. Denervated muscle is known to be more permeable than normal muscle to potassium ions (26), and the permeability of normal muscle for magnesium is very low (see p. 405).

A comparison of electrical stimulation of the single endplate in the lizard with stimulation of its nerve showed that the neuromuscular transmission was reversibly blocked for nerve stimulation by 4–8 mM magnesium, whereas the response to direct stimulation of the endplate remained unaffected at concentrations up to 80 mM (42). Lizard muscle was found to be less susceptible to magnesium than frog muscle.

The blocking effect of magnesium at the neuromuscular junction is in certain respects similar to that of curare. Curare is known to depress the sensitivity of the muscle to intra-arterially injected acetylcholine (21) and to acetylcholine applied directly to the single endplate (24, 25, 80). Also magnesium produces a decreased sensitivity to the stimulating effect of acetylcholine on the single endplate (42). However, the site of action of magnesium and curare is presumably different, as magnesium blocks the stimulating effect of potassium as well. This was examined on the sympathetic ganglion, in which magnesium blocked transmission and prevented the stimulating effect of acetylcholine and potassium, whereas curare blocked the response to acetylcholine, but not to potassium (126). The contracture produced by acetylcholine and by potassium in the frog's rectus abdominis was antagonized by magnesium, the necessary concentration being about 10 times less for potassium than for acetylcholine (71a). Whether magnesium also blocks the muscular contraction produced by potassium remains to be shown. Some further light might be thrown on the site of action of magnesium at the neuromuscular junction by electrophysiological methods. At present it is unknown whether magnesium depolarizes or stabilizes the membrane potential at the site of the endplate.

In crabs, in which the neuromuscular system is unaffected by acetylcholine and curare, raising of magnesium concentration about 2.5 times above the normal blood level produced a complete neuromuscular block, whereas higher magnesium concentrations were required to affect the muscle substance itself. The normal blood magnesium level in crabs (about 26 mM) was about half that present in sea water and is well above the level which blocks neuromuscular transmission in vertebrates. The electric response of the nerve was increased by magnesium. Potassium in concentrations about 3 times the normal blood level produced contracture, which was prevented by magnesium (78). The neuromuscular transmission was facilitated by perfusion with fluids containing 5–20 per cent of the normal blood concentration of magnesium (11). It seems that the blocking effect of magnesium is very widespread, and obtained even when the mechanism of neuromuscular transmission is different and not influenced by curare, supporting the view of different sites of action of the two substances.

Investigations of the effect of tetanic stimulation during progressive neuromuscular block caused by curare or magnesium point in the same direction (16, 90, 107, 108). By stimulation of the motor nerve of mammalian muscle *in situ* during partial curarization there is a brief phase of facilitation; when a single stimulus is blocked, two stimuli of the same strength in rapid succession may still excite. The facilitatory effect of a single volley is maximum after about 3 msec. During tetanic stimulation of the nerve this effect is found only with the first few stimuli; it is followed by a decrease in height of contraction, which occurs even with a series of twitches elicited at 10-second intervals. This implies that the inhibitory after-effect of the single stimulus has a minimum duration of 10 seconds in partially curarized muscles (113a). The decline in tension is the more marked, the higher the frequency of stimulation (frequency of stimulation 20–120 per sec.) (16). Moreover, during partial curarization tetanic stimulation through the nerve is followed by a period of post-tetanic potentiation, during which the response to a single volley is enhanced. This post-tetanic effect lasts several minutes depending on the duration and the frequency of the conditioning tetanus (14, 113a).

These three effects are also found during a partial block produced by magnesium but there are certain differences. Magnesium produces an early facilitation with a time course identical for both substances (90). However, the decrease in tension of contraction, which is pronounced after curare, is slight and transient during magnesium and, in contrast to findings with curare, an increase in stimulation frequency reduces the period during which the contractions are decreased (16). This was confirmed by Naess (107) who applied a somewhat different method of stimulation using periods of different stimulation frequencies (1–300 c.p.s.). He found that curare produced a pronounced inhibition to high stimulation frequencies, whereas contractions produced by single stimuli and low frequencies showed less reduction in amplitude. Magnesium produced inhibition at low stimulation frequencies, with comparatively little reduction in contraction amplitude at high frequencies (107). When the neuromuscular transmission was nearly blocked, neostigmine in curarized muscle could only restore the single twitches, whereas after magnesium it restored the response to all frequencies of stimulation (107, 108).

The post-tetanic effect found with curare was present with magnesium as well (88). The degree and the duration of the post-tetanic potentiation vary in the same way with the conditioning tetanus under magnesium and curare (15). But with magnesium the post-tetanic twitches are maximal immediately after the tetanus and decline gradually; with curare the amplitude of the first post-tetanic twitches is relatively low, and become progressively higher until a maximum is reached, which suggests that the post-tetanic potentiation is gradually unmasked (16).

The early short period of facilitation during partial curarization has been explained as being due to summation of the endplate potentials which reach threshold for the establishment of transmission of impulses (39). The time course of facilitation was approximately the same for curare and magnesium, which suggests the same mechanism of facilitation. The post-tetanic potentiation outlasts the stimulation of the nerve for several minutes, and it is improbable that acetylcholine can account for it. Rosenblueth and Morrison (113a) assumed that it might result from potassium liberated during stimulation of the nerve. That potassium actually can cause a potentiation similar to that after electrical stimulation of the nerve was shown by Brown and v. Euler (22). Potassium was assumed to lower the threshold of muscle fibers previously unexcitable. It is in accordance herewith that potassium can counteract curarization and antagonize the magnesium block (20).

An explanation for the decrease in contraction height found during tetanic nerve stimulation of the partially curarized muscle, and for the differences between curare and magnesium effects is still lacking. There are several hypotheses which deal with changes in the amount of acetylcholine released or changes in threshold (113a, 113, 90), but none seems fully satisfactory. That neostigmine after curarization reestablishes only single twitches but not a response to high frequency stimulation indicates a complex mode of action of curarine (108).

Cardiovascular System. The depressant action of magnesium on the heart was known before Meltzer and Auer's studies stimulated interest in the anesthetic action and demonstrated that the cause of death after parenteral administration was respiratory failure. That magnesium causes a fall in blood pressure was demonstrated by Meltzer and Auer (96) and later confirmed by several authors (94, 133, 93). The main interest of recent investigators was an attempt to find the site of the complicated action on the cardiovascular system.

Electrocardiographic examinations demonstrated a depressant action on rhythm and conduction. Magnesium prolonged the P-Q conduction time and extended the QRS complex, while the cardiac rate was lowered (100, 114, 101, 122).

The action upon the conduction system of the heart was shown to be independent of vagus impulses (34). More recently it was demonstrated that slowing of the heart rate is caused partly by blocking of the cardioaccelerator ganglion (see p. 403), but in addition there is a direct inhibitory action on the spontaneous rhythm of the heart (126). At a time when the conduction system was affected, the heart muscle itself was relatively unimpaired, the systole being vigorous until the heart stopped (122). Mechanical stimulation was still possible after cardiac arrest (114). This is in agreement with recent investigations on the effect of magnesium on the isolated papillary muscle. Calcium and magnesium produced a slight rise in threshold, the effect of magnesium was least pronounced and very small compared with the effect of potassium. Magnesium had no demonstrable effect on either myogram or electrogram (61, 56). Direct investigations on the heart-lung preparations demonstrated that increase of the magnesium concentration in serum diminished the competence of the heart and decreased the systemic output. The total output remained nearly constant and, because of the decrease in rate, the stroke volume was increased (127). Competence of the heart could be restored by calcium, which is in contrast to the findings of Smith, Winkler and Hoff (122), who found that the serum magnesium concentration necessary to stop the heart action was not increased by addition of calcium.

The serum concentrations associated with depression of the cardiac conduction system were examined by Smith, Winkler and Hoff (122). After a transient accelerating effect (probably produced by the fall in blood pressure caused by magnesium), the effect on conduction from auricles to ventricles occurred at approximately 3–5 mM magnesium, and cardiac arrest usually occurred within a serum magnesium range of 15–22 mM, the effective concentration depending on the rate of infusion. Cardiac failure occurred at considerably higher concentrations than those necessary for respiratory failure. In heart-lung preparations the rate of discharge of the sino-atrial node was progressively depressed by increasing concentrations of magnesium in the serum, a 10 per cent fall in heart rate being found with a serum concentration of about 4 mM of magnesium (127). This is in good agreement with findings in intact animals and indicates that the slowing effect on heart rate begins at about the same concentration as the depressant action on the reflexes.

Magnesium has especially been claimed to counteract extranodal cardiac rhythms arising spontaneously or during therapy with digitalis glycosides. In digitalized dogs, magnesium increased the degree of block by producing a further delay in auriculo-ventricular conduction time and produced impulses of ectopic origin (102). In dog heart-lung preparations, cardiac irregularity and fatal doses of digoxin were not altered by increasing serum magnesium concentrations (up to approximately 10 mM). But ventricular irregularities caused by digoxin could be temporarily eliminated by injection of large amounts of magnesium solution in the heart-lung preparation, in normal dogs and in dogs with denervated hearts. The serum magnesium concentration was determined in one case to be approximately 4 mM magnesium when the rhythm returned to normal (127). The explanation of these discrepancies is not clear. The authors suppose that massive doses temporarily produce a concentration of magnesium in the coronary circulation which may be sufficient to stop foci of origin of heterotopic rhythms, but which would be lethal if maintained over a long period.

The initial depressing action on the *blood pressure* occurs at very low serum magnesium concentrations (1–3 mM magnesium) (72), and is accompanied by a pronounced vasodilatation. As the serum concentration of magnesium is gradually increased, the fall in blood pressure continues until the heart stops. During more recent years, interest was concentrated on the possible mechanisms responsible for the blood pressure lowering effect of magnesium. The main factor seems to be a *blocking action on the sympathetic ganglia.* If magnesium is given during stimulation of the cervical sympathetic nerve, a brief relaxation of the nictitating membrane is observed. The principal site of this activity is located in the ganglion, since concentrations which block the ganglionic potential and ganglionic transmission have no influence on the conduction of impulses in pre- and post-ganglionic fibers. A blockage of impulse transmission by magnesium was found for the superior cervical, stellate and inferior mesenteric ganglia. This general blocking effect on sympathetic ganglia is assumed to be an important factor in the blood pressure lowering effect of magnesium (126). As stressed by Stanbury (126) other factors might be involved as well. (a) An effect on the vasomotor center; (b) a direct effect on peripheral vessels; and (c) change in cardiac output. A depressant effect on the *vasomotor center* was considered unlikely by this author, since the intracarotid injection of magnesium produced a *rise* in blood pressure even after exclusion of vasomotor reflexes by evulsion of the cranial nerves IX through XII (126). Perfusion of the cerebral ventricles with a solution containing an excessive magnesium concentration (approximately 10 mM magnesium), caused a fall in arterial pressure and a depression of the vasomotor reflexes. This effect of magnesium was similar to that of calcium but less pronounced (84), and its central origin can hardly be doubted. The concentrations which evoke this depression are rather high as compared with those required for neuromuscular block. A depression of carotid vasomotor reflexes could be produced by magnesium injection and was antagonized by calcium (68a). By the technique applied, the site of action could not be decided. It is not known whether calcium antagonizes the blocking effect of magnesium on sympathetic ganglia.

A *direct effect on peripheral vessels* can hardly be excluded. Magnesium produced a fall in blood pressure in spinal dogs, and a vasodilatation was observed in frogs with the central nervous system destroyed (66). This was confirmed on spinal cats and in a cat in which sympathetic tone was eliminated by stabilizing the blood pressure at a high level, injection of magnesium still causing a fall in blood pressure (126). Stanbury (126) emphasizes that it is not possible on the basis of these observations to differentiate between an effect on the cardiac output and a direct effect on peripheral vessels.

As to a decrease in *cardiac output* as the cause of the fall in blood pressure produced by magnesium, experiments on isolated heart-lung preparations demonstrated a decrease in cardiac competence, but the total output and the mean arterial pressure remained very nearly constant during successive additions of magnesium chloride (concentrations of the order of magnitude of 7 mM magnesium) (see p. 402). It seems improbable, therefore, that depression of the heart plays an important role in the lowering of blood pressure after magnesium in anesthetic concentrations.

It may be concluded that the depressant action of magnesium on the blood pressure is complicated; hardly any of the possible mechanisms can be excluded;

most important is its blocking effect on sympathetic tonus, but still more experimental work is needed to clear up the relative influence of the individual mechanisms of action.

Smooth Muscle. A direct effect of magnesium on smooth muscle of peripheral vessels remains to be shown. It seems rather likely that it exists since there is a direct depressant effect on other smooth muscles. A direct inhibitory action on the nictitating membrane was found in addition to the inhibition produced by blocking the superior cervical ganglion (126). A bronchodilator action of magnesium was found by perfusion of excised guinea pig lungs (65). In isolated intestine, magnesium chloride reduced the contraction height obtained with acetylcholine and decreased the sensitivity of the muscle (140). In uterine muscle, an increase in magnesium concentration above that of the usual Ringer's solution increased sensitivity to oxytocic hormone, whereas it interrupted or prevented the spontaneous rhythm. This effect has been employed in the biological assay of oxytocin (53, 74).

Antagonists to the Depressant Action of Magnesium. The depressant action of magnesium is to a large extent antagonized by an excess of calcium. At moderate magnesium doses intravenous administration of calcium acts immediately whereas the protective action of calcium fails at high magnesium doses (97). Calcium counteracts both the central and the peripheral depression (22a). Eserine can counteract the effect of magnesium as well, but it acts more slowly (128) and the depressant action is not completely abolished. With eserine the effect on respiration is said to be the most prominent (76). The depressant action of magnesium on the neuromuscular junction is antagonized by neostigmine but not so completely as by calcium (91). A combination of pentamethylenetetrazol and neostigmine antagonizes magnesium anesthesia nearly completely, whereas pentamethylenetetrazol alone has a slight effect and only on respiration. Neostigmine alone has a somewhat more pronounced effect than pentamethylenetetrazol, but still significantly less than the combined action of the two drugs. The magnesium depression of indirect excitability of the muscle was unaffected by pentamethylenetetrazol, but counteracted by neostigmine. The interpretation of these findings is in agreement with the assumption of both a central and a peripheral site of action of magnesium, the former being antagonized by the analeptic, neostigmine affecting the peripheral point of attack, and calcium intervening in both (13).

Permeability. The effect of magnesium on the neuromuscular system is closely related to its concentration in the serum (45, 73, 104, 42), but the concentration in the central nervous system and the muscles does not increase during magnesium anesthesia. This was first demonstrated in brains of dogs dying under magnesium anesthesia (92), and was later confirmed on brain and muscle (45, 131). In more recent experiments, a slight increase of magnesium content was found in frog and mammalian muscle; this, however, was small as compared with that of serum and it might be explained by an increase of the magnesium in the extracellular space only (29, 130).

The lack of proportionality between the magnesium concentration in blood and in tissue is presumably due to the relative impermeability of the cellular membrane to magnesium. When magnesium is administered intravenously in the intact animal, it will be distributed over 20 to 25 per cent of the body weight during the first 4 hours. This volume of distribution corresponds approximately to the volume of the extracellular space. Later, within 24 hours, but at a time when the anesthetic effect has ceased, a variable amount, which cannot be accounted for as having been excreted, will leave the extracellular space and be deposited in an unknown part of the body (124). Perfusion of the hindlegs of frogs indicates that magnesium in this preparation can penetrate cells (muscle or bone), the volume of distribution being greater than that of the extracellular space (52). Whether this discrepancy is due to differences between intact and perfused muscles, or whether there is some penetration of magnesium even in the former can not be decided at present. Experiments on excised tissues have only been performed on frog muscle and show a slow penetration of magnesium. Suspension of frog muscle in solutions containing 80 mM magnesium showed a rapid increase in magnesium concentration during the first half hour, followed by a slow constant increase. Determinations of the extracellular space by means of magnesium and inulin during the first half hour both gave 9 to 10 ml per 100 gram of muscle; hence, only the slow increase of magnesium concentration was due to actual penetration into the muscle cell (18). If isolated frog muscle is exposed for a long time (5 hours) to magnesium concentrations of the same order of magnitude as those found during anesthesia (above 4 mM) magnesium enters the muscle cell. The calculated volume of distribution was 60 per cent or more, indicating that magnesium slowly penetrates the muscle cell (52).

Experiments with radioactive isotopes of magnesium have not been performed as half-life of Mg^{23} and Mg^{27} unfortunately is very short (11.6 sec. and 9.6 min., respectively).

The magnesium content of the central nervous system and of muscle is high compared with the concentration in plasma. The concentration of magnesium in the central nervous system is about 4.5 to 6 mM/kg (45, 29, 41), in muscle about 8 to 12 mM/kg (30, 51, 29), in plasma about 1.2 to 1.6 mM/kg. The figures show some variation in different species and different individuals, and with different methods of assay, but the order of magnitude is the same. Although by far the greater portion of intracellular magnesium in muscle is unionized, the actual concentration of the ionized and the unionized fractions is unknown. A frog muscle suspended in magnesium-free Ringer's solution retains 80 to 90 per cent of the total magnesium up to the fifth day (31). The main loss of magnesium seems to occur within the first 5 hours, during which the loss amounts to about 14 per cent of the total magnesium content (52). It is uncertain to which anions magnesium is bound within the cell. The finding that the magnesium content of muscle cells is relatively constant in magnesium deficiency is consistent with the assumption that magnesium is mainly bound to protein and organic phosphate anions (135, 32).

Histochemical assay of magnesium in striated muscle fiber has given rather contradictory results. It is well established that micro-incineration of striated muscle gives ash diagrams with a distribution which resembles the cross striation. It is, however, difficult to decide whether the band with high ash content corresponds to the anisotropic or to the isotropic segment of the fiber. Comparing a micro-incinerated and an adjacent stained section in mammalian muscle, Scott (117, 118) concluded that the ash lay in the anisotropic segments. In insect muscle Engström (44) compared the site of the main ultraviolet absorption and of the ash content in the same fiber in relation to well-defined reference points. In these experiments, the ash was found to be localized in the ultraviolet absorbing isotropic segments, while the anisotropic segments were practically devoid of ash. By application of emission electron microscopy it was possible to differentiate magnesium and calcium from potassium and sodium. Magnesium and calcium were present almost exclusively within the muscle, but it was uncertain where in relation to the anisotropic and isotropic segments (119).

By use of the electron beam of the electron microscope for micro-incineration, not only organic but also more volatile inorganic material is removed and the cross-striation disappears. The narrow striations of the fibril with a period of 400 A are, however, present. This periodicity is interpreted as being due to the more stable salts of calcium and magnesium (37). This would suggest that the magnesium and calcium are distributed independently of the anisotropic and isotropic segments. It seems, however, that the interpretations of the findings need further experimental support.

Mechanism of Action. Anesthesia with magnesium is the only example of anesthesia produced by a metallic electrolyte. When attempts are made to explain its mode of action, it seems natural to consider its role in the enzymatic processes considered essential for the function of the neuromuscular system. The magnesium ion has been shown to be an activator for tissue phosphatases and phosphate transferring enzymes. Its presence is of importance for the activity of several enzymes with special functions in the neuromuscular system. The best known of these are the enzymes which cause the enzymatic breakdown of adenosinetriphosphate, and the enzymes which synthesize and split acetylcholine in tissue. However, the main difficulty for all interpretations is that the magnesium ion penetrates the cell slowly, while its depressant action occurs soon after an intravenous injection and is abolished almost instantaneously by calcium. Consequently it has often been suggested that the site of the anesthetic action of magnesium ion must be primarily at the cell membrane, especially at that of the neuromuscular junction. This is in accordance with the small increase in magneseium concentration in brain and muscle found during magnesium anesthesia.

According to current concepts, the following sequence of events occurs in neuromuscular transmission: The arrival of the nerve impulse with subsequent liberation of acetylcholine, which depolarizes the endplate; the depolarization initiates a propagated impulse in the muscle fiber. Accordingly several mechanisms could be involved in the blocking action of magnesium: the acetylcholine production might be reduced; the endplate membrane might be prevented from responding normally; or the excitability of the muscle fiber membrane or the contractility of the fiber substance might be decreased. The magnesium effect seems primarily to be localized to the endplate membrane, as the sensitivity to acetylcholine was found to be decreased while the excitability of the muscle was still unimpaired. Sooner or later the muscle fiber is also affected. At the moment, it is solely a matter for speculation whether magnesium, like curarine, decreases the endplate potential, depolarizes it or acts by some other unknown mechanism. As mentioned before, the experimental evidence seems to indicate a mechanism of action which is different from that of curarine.

The explanation of the antagonistic effect of calcium is likewise obscure, though a little more is known about the effect of calcium on the endplate potential. Calcium produces a more general decurarizing effect, as curarization likewise was found to be counteracted by calcium (50). Excess of calcium (4 to 5 times normal content) increased the size of the endplate potential in curarized frog muscle without appreciably altering its time course. Calcium raised the threshold potential required for the initiation of muscle spikes with a given concentration of curarine (40, 79, 28). In isolated fibers from frog muscle, a moderate excess of calcium first caused a delay in onset of the spike, which indicates a decrease in excitability of the muscle fiber. The amplitude of the endplate potential was not increased by calcium. A further increase in the calcium concentration caused neuromuscular block (81). The decurarizing effect of calcium has been explained as being due to increased liberation of acetylcholine (33). In curarized muscle, in contrast to normal, excess calcium increased the amplitude of the endplate potential. This increase was not accompanied by an increase in sensitivity to the depolarizing action of acetylcholine in the endplate region. This suggests that the increase in amplitude may be due to an increase in the amount of acetylcholine released at the endplate (28).

It is difficult to explain the magnesium-calcium antagonism on the basis of these findings. We do not know the influence of calcium excess on the endplate potential in a magnesium-treated muscle; and if calcium produces an increased acetylcholine release this might be accompanied by a decrease in excitability of the muscle fiber.

At higher concentrations of magnesium, a decrease in excitability and contractility of the muscle develops slowly, probably produced by the slowly penetrating magnesium. As mentioned above, magnesium ion influences the enzymatic breakdown of adenosinetriphosphate (ATP), at present believed to be the final link between the energy-yielding chemical reactions and mechanical activity.

The finding that myosin acts as an ATP-ase (43) links the process of energy transfer to the contractile structure. The enzymatic activity of myosin and actomyosin (3, 132) was found to be strongly influenced by the electrolyte medium; among other ions it was activated by calcium (2), whereas magnesium inhibited ATP-ase activity (86). The ATP-ase activity of myosin and actomyosin was differently influenced by magnesium. Pure myosin was inhibited at even the lowest concentrations of magnesium, whereas phosphatase activity of actomyosin was enhanced by magnesium with an optimum between 1 and 5 mM magnesium. At high ionic strength (100 mM KCl), the activity of actomyosin was inhibited by all magnesium concentrations, which was explained as being due to a dissociation of the protein complex into actin and myosin. Calcium strongly enhanced the enzymatic activity at concentrations between 1 and 100 mM; this enhancing effect was inhibited by all concentrations of magnesium (4). The phosphate uptake of actomyosin threads with low ATP-ase activity was enhanced by magnesium (100 mM) (23).

The antagonistic action of magnesium-calcium in muscle was confirmed by Greville and Lehmann (62) who tried to correlate it to the magnesium-calcium antagonism of magnesium anesthesia. The rôle of magnesium ions in enzymatic processes in living muscle is, however, complicated by its dependence on several factors which are not sufficiently elucidated: the mutual relation between actomyosin and myosin, intramuscular pH, ionic strength and the concentration of other activators and inhibitors. As mentioned before, the degree of ionization and the localization of magnesium in the fiber are uncertain and several contradictory statements about the effect of magnesium ions are met with. On the basis of a study of the magnesium-calcium antagonism under a variety of conditions it was concluded that myosin ATP-ase under the conditions met with in muscle is probably subjected to the inhibitory action of magnesium (103). On the other hand, ATP-ase activity of isolated myofibrosils (which retained considerable organisation of structure) was found to be activated by magnesium (maximum of activation at 5 mM) even in the presence of 100 mM KCl. Destruction of structural organisation by 1.0 M KCl or by storing transformed the magnesium activation to inhibition. Evidence was presented that the ATP-splitting enzyme was actomyosin, and it was presumed that the state of organisation of actomyosin in the fibrils was responsible for this difference in behaviour (111). In view of these discrepancies and of our incomplete knowledge of the conditions for enzymatic activity in living muscle fiber, it seems hardly possible at present to give a consistent explanation for the depressant action of magnesium on muscle.

The question of the effect of magnesium on ATP-ase activity in living tissues was treated from another point of view by examining the amount of ATP in muscle and in brain during magnesium anesthesia. The ATP gain was higher in muscle and in brain samples taken during magnesium anesthesia than in those from the unanesthetised animal. There was a difference between animals in ether anesthesia and in magnesium anesthesia as well, which was claimed to indicate that the decrease in ATP-content was not solely caused by the increased muscular activity in unanesthetised animals. The increased content of ATP found in muscles after application of magnesium was interpreted as being due to magnesium inhibition of the enzymatic breakdown at ATP into ADP and inorganic phosphate (38). These findings in muscle were confirmed by Stoner (130) who, on the basis of experiments with P^{32}, interpreted the differences in another way. The specific activity (counts per min. per mg P) of (ATP)P and phosphocreatine P in magnesium-treated animals was significantly higher than in the controls, whereas the specific activity of total organic P and the sum of (ATP)P acid and stable ester P were unaltered. It was suggested that magnesium reduces the utilization of ATP and of phosphocreatine in glycolysis and leads to an accumulation of high energy phosphate bonds. That magnesium anesthesia increases the ATP content of the muscle is well established, but more experimental work is needed to determine the mechanisms involved. Experiments on curarized animals could possibly be of assistance in clarifying how far the increased amount of ATP in muscle after magnesium is due to the absence of muscular movements.

A group of enzymes which are of great importance for the special functions of the neuromuscular system and which are affected by the magnesium ion are acetylcholinesterase and acetylcholinacetylase.

The acetylcholine-splitting enzymes are known to be activated by magnesium ions. Most investigations are concerned with the non-specific cholinesterase (98, 54, 55), but even the specific acetylcholinesterase from the electric organ of torpedo was activated by magnesium in physiological concentrations, up to 2 mM. Calcium likewise acted as an activator (105). It would be interesting to know whether the concentrations obtained during magnesium anesthesia also produce activation of cholinesterase, as an increase in the enzymatic breakdown of acetylcholine would inhibit neuromuscular transmission as well. The antagonistic effect of calcium cannot, however, be explained by the effect on cholinesterase.

Acetylcholine can be synthesised in cell-free solution. The enzyme responsible for the synthesis was termed choline acetylase (106). This synthesis was diminished by calcium ions (49) and activated by magnesium ions in the presence of citrate, the optimal concentration of magnesium being 4 mM (47, 48). As the concentration of magnesium in the central nervous system is about 5 mM, it seems plausible that an increase in the concentration of magnesium ions in the extracellular fluid might inhibit the synthesis of acetylcholine. Experiments on the cholineacetylase activity of brain tissue during magnesium anesthesia might contribute to the solution of this problem.

Assay of Magnesium. The earliest method for the determination of magnesium in biological fluids is based upon its precipitation as magnesium ammonium phosphate. Magnesium is determined as phosphate by reduction of phosphomolybdic acid to a blue substance, the amount of which is measured colorimetrically (19, 35, 99, 124). Moreover, phosphate can be determined as the yellow molybdivanadophosphoric acid, which is claimed to be stable over a longer time than the molybdenum blue (120, 121).

Another principle for the determination of magnesium is precipitation with 8-hydroxyquinoline. The hydroxyquinoline bound to magnesium can be determined in several ways:

Bromination of the magnesium hydroxyquinoline was described by Greenberg and Mackey (60), the chief source of error being a loss of bromine through evaporation (59). A cerimetric titration of the hydroxyquinoline has the advantage, that the amount of ceric sulphate consumed by the same amount of hydroxyquinoline is about 8 times greater than that of bromate in bromatometric titration (125). Colorimetric determination may be used after addition of diazotised sulphanilic acid (112). Another possibility is to measure the hydroxyquinoline micromanometrically by direct combustion of the hydroxyquinoline complex, the carbon being determined as CO_2. This method was claimed to be more sensitive than the colorimetric methods since the precipitate contains 18 carbon atoms per atom of Mg (70). The formation of the insoluble Mg-hydroxyquinolate is also the basis for the polarographic determination of magnesium. Direct polarographic determination is impossible, but the excess of oxyquinoline left after precipitation with magnesium is determined by this technique (129, 136).

Before the determination of magnesium with ammonium phosphate or hydroxyquinoline it is necessary to precipitate the calcium. One of the main difficulties in the removal of calcium as oxalate is the separation of magnesium and calcium, as some magnesium oxalate is always occluded. The difficulty arises chiefly when the quantity of magnesium exceeds that of calcium (8, 12). In normal serum the separation of calcium and magnesium apparently has not presented difficulties for the estimation of magnesium. In magnesium anaesthesia, when the serum contains about 10 times the value of the normal serum, the separation of calcium results in a loss of 4 to 8 per cent of the total magnesium (42). This error could be overcome by using slow precipitation at high temperature (100°C.) (8) at low p*H* (3 to 4) (89, 137).

Several methods exist which can be used in the presence of calcium. The colorimetric method for the determination of magnesium by means of Titan yellow is based on the development of a red magnesium hydroxide-titan yellow complex (6, 64, 69, 82). With this method calcium need not be removed, since it does not interfere with the colour produced by magnesium in concentrations ordinarily found in blood (57). The sensitivity of the method was increased 10-fold by Orange and Rhein (109). This method might prove suitable with larger amounts of magnesium in plasma as well.

In addition to precipitation, magnesium in body fluids can be determined by direct spectroscopy (17, 27).

A method for the determination of magnesium based upon isolation by electro-deposition in the presence of calcium has been developed by Terkildsen (134).

REFERENCES

(1) Ashkenaz, E. W. Magnesium narcosis in muscle. J. Cell. & Comp. Physiol., **11:** 163–174, 1938.

(2) Bailey, K. Myosin and adenosinetriphosphatase. Biochem. J., **36:** 121–139, 1942.

(3) Banga I. and Szent-Györgyi, A. Preparation and properties of myosin A and B. Stud. Inst. Med. Chem. Univ. Szeged, **1:** 5–15, 1942.

(4) Banga, I. and Szent-Györgyi, A. The influence of salts on the phosphatase action of myosin. Stud. Inst. Med. Chem. Univ. Szeged, **3:** 72–75, 1943.

(5) Baumecker, W. Experimentelle Beiträge zum Antagonismus der Magnesium- und Calciumionen. Biochem. Ztschr., **142:** 142–158. 1923.

(6) Bécka, J. Über die praktische Anwendung der kolorimetrischen Magnesiumbestimmung in der Biochemie. Biochem. Ztschr. **233:** 118–128, 1931.

(7) Beecher, H. K., McDonough, F. K. and Forbes, A. Effects of blood pressure changes on cortical potentials during anesthesia. J. Neurophysiol., **1:** 324–331, 1938.

(8) Benedetti-Pichler, A. Microversuche über quantitative Trennungen. 1. Mitteilung. Versuche unter Benutzung des Filterstäbschens. Ztschr. f. analyt. Chemie, **64:** 409–436, 1924.

(9) Bertrand, J., Gayet-Hallion, Th. and Quivy, D. Influence des seis de magnésium sur l'electro-encéphalogramme du Lapin. Compt. rend. Soc. biol., **141:** 145–147, 1947.

(10) Binet, P. Recherches comparatives sur l'action physiologique des metaux alcalins et alcalino-terreux. Rev. méd. Suisse Rom., **12:** 523–550, 587–611, 1892.

(11) Boardman, D. L. and Collier, H. O. J. The effect of magnesium deficiency on neuro-muscular transmission in the shore crab, carcinus maenas. J. Physiol., **104:** 377–383, 1946.

(12) Bobtelsky, M. and Malkowa-Janowski. Über die Löslichkeit von Magnesiumoxalat und ihre Bedeutung für die Calcium-Magnesium Trennung. Ztschr. f. angew. Chemie, **40:** 1434–1436, 1927.

(13) Borglin, N. E. and Lindstén, T. On the combined effect of neostigmine and pentamethylenetetrazol or β-phenylisopropylamine on magnesium anaesthesia. Acta pharmacol. et toxicol., **5:** 309–320, 1949.

(14) Boyd, T. E. Recovery of the tongue from curare paralysis, following prolonged stimulation of the hypoglossal nerve. Am. J. Physiol., **100:** 569–575, 1932.

(15) Boyd, T. E. and Brosnan J. J. Decurarizing effect of repetitive stimulation of a motor nerve. Proc. Soc. Exper. Biol. & Med., **35:** 404–405, 1936.

(16) Boyd, T. E. Brosnan, J. J. and Maaske, C. A. The summation of facilitating and inhibitory effects at the mammalian neuromuscular junction. J. Neurophysiol. **1:** 497–507, 1938.

(17) Boyle, A. J., Whitehead, T., Bird, E. J., Batchelor, Th. M., Iseri, L. T., Jacobson, S. D. and Myers, G. B. The use of the emission spectrograph for the quantitative determination of Na, K, Ca, Mg, and Fe in plasma and urine. J. Lab. & Clin. Med., **34:** 625–630, 1949.

(18) Boyle, P. J., Conway, E. J., Kane, F. and O'Reilly, H. L. Volume of interfibre spaces in frog muscle and the calculation of concentrations in the fibre water. J. Physiol., **99:** 401–414, 1941.

(19) Briggs, A. P. A colorimetric method for the determination of small amounts of magnesium. J. Biol. Chem., **52:** 349–355, 1922.

(20) Brosnan, J. J. and Boyd, T. E. Agents which antagonize the curare-like action of magnesium. Am. J. Physiol. **119:** 281–282, 1937.

(21) Brown, G. L., Dale, H. H. and Feldberg, W. Reactions of the normal mammalian muscle to acetylcholine and to eserine. J. Physiol., **87:** 394–424, 1936.

(22) Brown, G. L. and von Euler, U. S. The after effect of a tetanus on mammalian muscle. J. Physiol., **93:** 39–60, 1938.

(22a) Bryant, G. W., Lehmann, G. and Knoefel, P. K. The action of magnesium on the central nervous system and its antagonism by calcium. J. Pharmacol. & Exper. Therap., **65:** 318–321, 1939.

(23) Buchthal, F., Deutsch, A., Knappeis, G. G., and Munch-Petersen, A. Phosphorylation and adenine nucleotide uptake of actomyosin and actin-free myosin. Acta physiol. Scandinav., **16:** 326–344, 1949.

(24) Buchthal, F. and Lindhard, J. Direct application of acetylcholine to motor end plates of voluntary muscle fibres. J. Physiol., **90:** 82P–83P, 1937.

(25) Buchthal, F. and Lindhard, J. Transmission of impulses from nerve to muscle fibre. Acta physiol. Scandinav., **4:** 136–148, 1942.

(26) Cannon, W. B. and Rosenblueth, A. The supersensitivity of denervated structures. A law of denervation. The Macmillan Co., New York, 1949, p. 245.

(27) Cassen, B. A method for rapid determination of magnesium in body fluids and some preliminary results on clinical material. J. Lab. & Clin. Med., **25:** 411–413, 1940.

(28) Castillo, J. del and Stark, L. The effect of calcium ions on the motor end-plate potentials. J. Physiol., **116:** 507–515, 1952.

(29) Cloetta, M., Fischer, H. and van der Loeff, M. R. Die Verteilung und die Wirkung des Magnesiums im Organismus und deren Beeinflussung durch Calcium. Arch. exper. Path. u. Pharmacol., **200:** 6–42, 1942–1943.

(30) Conway, E. J. The physiological significance of inorganic levels in the internal medium of animals. Biol. Rev. **20:** 56–72, 1945.

(31) Conway, E. J. and Cruess-Callaghan, G., Magnesium and chloride "permeations" in muscle. Biochem. J., **31:** 828–836, 1937.

(32) Cotlove, E., Holliday, M. A., Schwartz, R., and Wallace, W. M. Effect of electrolyte depletion and acid-base disturbance on muscle cations. Am. J. Physiol., **167:** 665–675, 1951.

(33) Cowan, S. L. The actions of eserine-like compounds upon frog's nerve-muscle preparations, and conditions in which a single shock can evoke an augmented muscular response. Proc. Roy. Soc., London, s. B., **129:** 356–391, 1940.

(34) Dellen, T. R. Van and Miller, J. R. Electrocardiographic changes following the intravenous administration of magnesium sulfate. II. An experimental study on dogs. J. Lab. & Clin. Med., **24:** 840–843, 1939.

(35) Denis, W. The determination of magnesium in blood, plasma and serum. J. Biol. Chem., **52:** 411–415, 1922.

(36) Desprès, M. Action curarisante du chlorure de magnésium. Compt. rend. Soc. biol., **107:** 602–604, 1931.

(37) Draper, M. H. and Hodge, A. J. Sub-microscopic localization of minerals in skeletal muscle by internal "micro-incineration" within the electron microscope. Nature, London, **163:** 576–577, 1949.

(38) Dubois, K. P., Albaum, H. G. and Potter, V. R. Adenosine triphosphate in magnesium anesthesia. J. Biol. Chem., **147:** 699–704, 1943.

(39) Eccles, J. C., Katz, B. and Kuffler, S. W. Nature of the "endplate potential" in curarized muscle. J. Neurophysiol., **4:** 362–387, 1941.

(40) Eccles, J. C., Katz, B., and Kuffler, S. W. Effect of eserine on neuromuscular transmission. J. Neurophysiol., **5:** 211–230, 1942.

(41) Eichelberger, L., Kollros, J. J. and Walker, A. E. Water, nitrogen and electrolyte content of brain following cerebral concussion. Am. J. Physiol., **156:** 129–136, 1949.

(42) Engbaek, L. Investigations on the course and localization of magnesium anesthesia. A comparison with ether anesthesia. Acta pharmacol. et toxicol., **4:** Suppl. 1, Pp. 189, 1948.

(43) Engelhardt, W. A. and Ljubimowa, M. N. Myosine and adenosine-triphosphatase. Nature, London, **144:** 668–669, 1939.

(44) Engström, A. The localization of mineral salts in striated muscle fibers. Acta physiol. Scandinav., **8:** 137–151, 1944.

(45) Eskelund, V. Undersøgelser over magniumnarkosen og forholdet mellem magnium og calcium ved denne og nogle andre narkoser. Diss. Copenhagen 1931. Pp. 130.

(46) Essen, K. W. Untersuchungen über die Narkosevertiefung nach Grosshirnexstirpation. Arch. exper. Path. u. Pharmakol., **159:** 387–400, 1931.

(47) Feldberg, W. and Hebb, C. Effect of magnesium ions on the enzymic formation of acetylcholine. J. Physiol., **104:** 42P, 1945–1946.

(48) Feldberg, W. and Hebb, C. The effects of magnesium ions and of creatine phosphate on the synthesis of acetylcholine. J. Physiol., **106:** 8–17, 1947.

(49) Feldberg, W. and Mann, T. Formation of acetylcholine in cell-free extracts from brain. J. Physiol., **104:** 8–20, 1945.

(50) Feng, T. P. Studies on the neuromuscular junction. I. The inhibition at the neuromuscular junction. Chinese J. Physiol., **10:** 417–434, 1936.

(51) Fenn, W. O. Electrolytes in muscle. Physiol. Rev., **16:** 450–487, 1936.

(52) Fenn, W. O. and Haege, L. F. The penetration of magnesium into frog muscle. J. Cell. & Comp. Physiol. **19:** 37–46, 1942.

(53) Fraser, A. M. The effect of magnesium on the response of the uterus to posterior pituitary hormones. J. Pharmacol. & Exper. Therap., **66:** 85–94, 1939.

(54) Frommel, E., Herschberg, A. D. and Piquet, J. Effets des ions inorganiques sur l'activité de la cholinestérase sérique. I. Etudes, in vitro, sur le sérum de cheval. Helvet. physiol. et pharmacol. acta, **2:** 169–191, 1944.

(55) Frommel, E., Herschberg, A. D. and Piquet, J. Effets des ions inorganiques sur l'activité de la cholinestérase sérique. II. Etudes, in vivo, sur le cobaye. Helvet. physiol. et pharmacol. acta, **2:** 193-201, 1944.

(56) Garb, S. The effects of potassium, ammonium, calcium, strontium and magnesium on the electrogram and myogram of mammalian heart muscle. J. Pharmacol. & Exper. Therap., **101:** 317-326, 1951.

(57) Garner, R. J. Colorimetric determination of magnesium in plasma or serum by means of Titan yellow. Biochem. J., **40:** 828-831, 1946.

(58) Greenberg, D. M. Mineral metabolism. Calcium, magnesium, and phosphorus. Ann. Rev. Biochem., **8:** 269-300, 1939.

(59) Greenberg, D. M., Anderson, C. and Tufts, E. V. A note on a closed titration flash for use in the bromometric determination of magnesium with 8-hydroxyquinoline. Application to the estimation of magnesium in the tissues and urine. J. Biol. Chem., **111:** 561-565, 1935.

(60) Greenberg, D. M. and Mackey, M. A. The determination of magnesium in blood with 8-hydroxyquinoline. J. Biol. Chem., **96:** 419-429, 1932.

(61) Greiner, Th. H. and Garb, S. The influence of drugs on the irritability and automacity of heart muscle. J. Pharmacol. & Exper. Therap., **98:** 215-223, 1950.

(62) Greville, G. D. and Lehmann, H. Magnesium-calcium antagonism in muscle. Nature, London, **152:** 81-82, 1943.

(63) Guthrie, C. C. and Ryan, A. H. On the alleged specific anaesthetic properties of magnesium salt. Am. J. Physiol., **26:** 329-346, 1910.

(64) Haury, V. G. Modification of the Titan yellow method for the determination of small amounts of magnesium in biological fluids. J. Lab. & Clin. Med., **23:** 1079-1084, 1938.

(65) Haury, V. G. The broncho-dilator action of magnesium and its antagonistic action (dilator action) against pilocarpine, histamine and barium chloride. J. Pharmacol. & Exper. Therap., **64:** 58-64, 1938.

(66) Haury, V. G. The effect of intravenous injections of magnesium sulfate on the vascular system. J. Pharmacol. & Exper. Therap., **65:** 453-460, 1939.

(67) Haury, V. G. Variations in serum magnesium in health and disease: a review. J. Lab. & Clin. Med., **27:** 1361-1375, 1942.

(68) Hertz, H. Action potential and diameter of isolated fibres under various conditions. Acta physiol. Scandinav., **13:** suppl. 43, pp. 91, 1947.

(68a) Heymans, C. and Capet, L. Influences du magnésium et du calcium sur la regulation proprioceptive de la pression artérielle. Arch. internat. de pharmacodyn. et de thérap. **71:** 164-172, 1945.

(69) Hirschfelder, A. D. and Serles, E. R. A simple adaptation of Kolthoff's colorimetric method for the determination of magnesium in biological fluids. J. Biol. Chem., **104:** 635-645, 1934.

(70) Hoagland, C. Micromanometric determination of magnesium. J. Biol. Chem., **136:** 553-558, 1940.

(71) Hodgkin, A. L. and Katz, B. The effect of sodium ions on the electrical activity of the giant axon of the squid. J. Physiol., **108:** 37-77, 1949.

(71a) Hof, C. von and Schneider, H.-H. Über die Wirkungsweise von Magnesium im Gegensatz zu Curarin und Nikotin. Arch. exper. Path. u. Pharmakol., **214:** 176-184, 1952.

(72) Hoff, H. E., Smith, P. K. and Winkler, A. W. The relation of blood pressure and concentration in serum of potassium, calcium and magnesium. Am. J. Physiol., **127:** 722-730, 1939.

(73) Hoff, H. E., Smith, P. K. and Winkler, A. W. Effects of magnesium on the nervous system in relation to its concentration in serum. Am. J. Physiol., **130:** 292-297, 1940.

(74) Hsu, Yu-Chun. The influence of the magnesium concentration in the Ringer's solution used for the assay of oxytocin in pituitary (posterior lobe) extracts. J. Pharm. & Pharmacol., **21:** 146-151, 1948.

(75) Jolyet, F. and Cahours. Sur l'action physiologique des sulfates de potasse, de soude et de magnésie en injection dans le sang. Arch. physiol. norm. et path., **2:** 113-120, 1869.

(76) Joseph, R. and Meltzer, S. J. The life-saving action of physostigmin in poisoning by magnesium salts. J. Pharmacol. & Exper. Therap., **1:** 369-387, 1909-1910.

(77) Joseph, D. R. and Meltzer, S. J. Der hemmende Einfluss des Magnesiums auf die indirekte und direkte Erregbarkeit von Froschmuskeln und die antagonistischen Effekte von Natrium und Calcium auf diesen Einfluss. Zentralbl. Physiol., **24:** 7-8, 1910.

(78) Katz, B. Neuro-muscular transmission in crabs. J. Physiol., **87:** 199-221, 1936.

(79) Katz, B. Impedance changes in frog's muscle associated with electronic and "endplate" potentials. J. Neurophysiol., **5:** 169-184, 1942.

(80) Kuffler, S. W. Specific excitability of the endplate region in normal and denervated muscle. J. Neurophysiol., **6:** 99-110, 1943.

(81) Kuffler, S. W. The effect of calcium on the neuro-muscular junction. J. Neurophysiol., **7:** 17-26, 1944.

(82) Kunkel, H. O., Pearson, P. B. and Schweigert, B. A. The photoelectric determination of magnesium in body fluids. J. Lab. & Clin. Med., **32:** 1027-1033, 1947.

(83) Laget, P. and Lundberg, A. Further observations on environmental factors influencing the temperature sensitivity of mammalian nerve fibre. Effects of Ca^{++} and other agents. Acta physiol. Scandinav., **18:** 121-127, 1949.

(84) Leusen, I. The influence of calcium, potassium and magnesium ions in cerebrospinal fluid on vasomotor system. J. Physiol., **110:** 319-329, 1949.

(85) Libet, B. and Gerard, R. W. Control of the potential rhythm of the isolated frog brain. J. Neurophysiol., **2:** 153-169, 1939.

(86) Ljubimova, M. N. and Pevsner, D. Cit. from (2). Biokhimya, **6:** 178, 1941.

(87) Lorente de Nó, R. A study of nerve physiology. Part I, no. 131 Pp. 496. Rockefeller Inst. Med. Res., New York, 1947.

(88) Lubinska, L. Les troubles d'origine périphérique au cours de la narcose magnésienne. Arch. internat. de physiol., **41:** 456–473, 1935.

(89) Lux, H. Praktikum der quantitativen anorganischen Analyse. Berlin, 1941, p. 161.

(90) Maaske, C. A., Boyd, T. E. and Brosnan, J. Inhibition and impulse summation at the mammalian neuromuscular junction. J. Neurophysiol., **1:** 332–341, 1938.

(90a) Maaske, C. A. and Gibson, B. The effects of magnesium upon denervated mammalian muscle. Am. J. Physiol., **127:** 486–491, 1939.

(91) Malorny, G. and Ohnesorge, F. K. Magnesium-Kalzium Antagonismus bei Prüfung der neuromuskulären Erregbarkeit. Die Naturwissenschaften, **38:** 481–482, 1951.

(92) Mansfeld, G. and Bosányi, S. Untersuchungen über das Wesen der Magnesiumnarkose. Arch. f. d. ges. Physiol., **152:** 75–80. 1913.

(93) Markwalder, J. Experimentelle Untersuchung über Therapie des Wundstarrkrampfes mit intravenöser Magnesiuminfusion. Ztschr. f. d. ges. exper. Med., **5:** 150–172, 1917.

(94) Matthews, S. A. and Brooks, C. On the action of magnesium sulphate. J. Pharmacol. & Exper. Therap., **2:** 87–99, 1910.

(95) Meltzer, S. J. Magnesiumsulfat bei Tetanus. Berl. klin. Wchnschr., **52:** 261–266, 1915.

(96) Meltzer, S. J. and Auer. J. Physiological and pharmacological studies of magnesium salts. III. The narcotizing effect of magnesium salts upon nerve fibres. Am. J. Physiol., **16:** 233–251, 1906.

(97) Meltzer, S. J. and Auer, J. The antagonistic action of calcium upon the inhibitory effect of magnesium. Am. J. Physiol., **21:** 400–419, 1908.

(98) Mendel, B., Mundell, D. and Strelitz, F. Cholinesterase and electrolytes. Nature, London, **144:** 479–480, 1939.

(99) Michaels, G. D., Anderson, C. T., Margen, S. and Kinsell, L. W. A method for the colorimetric determination of calcium and magnesium in small amounts of urine, stool, and food. J. Biol. Chem., **180:** 175–180, 1949.

(100) Miki, Y. Experimentelle und klinische Untersuchungen über die Dauer des K-Ekg (Kammer-Elektrokardiogramms). Ztschr. f. d. ges. exper. Med., **27:** 323–388, 1922.

(101) Miller, J. R. and Van Dellen, T. R. Electrocardiographic changes following the intravenous administration of magnesium sulfate. An experimental study on dogs. J. Lab. & Clin. Med., **23:** 914–918, 1937–1938.

(102) Miller, J. R. and Van Dellen, T. R. Electrocardiographic changes following the intravenous administration of magnesium sulfate. III. Combined effect with digitalis. J. Lab. & Clin. Med., **26:** 1116–1120, 1940–1941.

(103) Mommaerts, W. F. H. M. and Seraidarian, K. A study of the adenosine triphosphatase activity of myosin and actomyosin. J. Gen. Physiol., **30:** 401–422, 1947.

(104) Moore, R. M. and Wingo, W. J. Blood level of magnesium ion in relation to the lethal, anesthetic, analgesic and antitetanic effects. Am. J. Physiol., **135:** 492–495, 1942.

(105) Nachmansohn, D. Action of ions on choline esterase. Nature, London, **145:** 513–514, 1940.

(106) Nachmansohn, D. and Machado, A. L. The formation of acetylcholine. A new enzyme: "choline acetylase". J. Neurophysiol., **6:** 397–403, 1943.

(107) Naess, K. The peripheral effects of magnesium and curare. Acta pharmacol. et toxicol., **8:** 137–148, 1952.

(108) Naess, K. The mechanism of action of curare. Acta pharmacol. et toxicol., **8:** 149–163, 1952.

(109) Orange, M. and Rhein, H. C. Microestimation of magnesium in body fluids. J. Biol. Chem., **189:** 379–386, 1951.

(110) Peck, C. H. and Meltzer, S. J. Anesthesia in human beings by intravenous injection of magnesium sulfate. J. Am. M. A., **67:** 1131–1133, 1916.

(111) Perry, S. V. The adenosinetriphosphatase activity of myofibrils isolated from skeletal muscle. Biochem. J., **48:** 257–265, 1951.

(112) Pignard, P. Microdosage colorimétrique du magnésium sanguin par la 8 hydroxyquinoléine. Bull. Soc. chim. biol., **32:** 401–408, 1950.

(113) Rosenblueth, A. The transmission of nerve impulses at neuroeffector junctions and peripheral synapses. John Wiley & Sons, Inc., New York, 1950, p. 325.

(113a) Rosenblueth, A. and Morison, R. S. Curarization, fatigue and Wedensky inhibition. Am. J. Physiol., **119:** 236–256, 1937.

(114) Rothberger, C. J. and Zwillinger, L. Über die Wirkung von Magnesium auf die Strophantin- und die Barium-Tachykardie. Arch. exper. Path. u. Pharmakol., **181:** 301–316, 1936.

(115) Rubin, M. A., Hoff, H. E., Winkler, A. W. and Smith, P. K. Intravenous potassium, calcium and magnesium and the cortical electrogram of the cat. J. Neurophysiol., **6:** 23–28, 1943.

(116) Schmidt, C. L. A. and Greenberg, D. M. Occurrence, transport and regulation of calcium, magnesium and phosphorus in the animal organism. Physiol. Rev., **15:** 297–434, 1935.

(117) Scott, G. H. Distribution of mineral ash in striated muscle cells. Proc. Soc. Exper. Biol. & Med., **29:** 349–351, 1932.

(118) Scott, G. H. The localization of mineral salts in cells of some mammalian tissues by micro-incineration. Am. J. Anat., **53:** 243–287, 1933.

(119) Scott, G. H. and Packer, D. M. An electron microscope study of magnesium and calcium in striated muscle. Anat. Rec., **74:** 31–45, 1939.

(120) Simonsen, D. G., Wertman, M., Westover, L. M. and Mehl, J. W. The determination of serum phosphate by the molybdivanadate method. J. Biol. Chem., **166:** 747–755, 1946.

(121) Simonsen, D. G., Westover, L. M. and Wertman, M. The determination of serum magnesium by the molybdivanadate method for phosphate. J. Biol. Chem., **169:** 39–47, 1947.

(122) Smith, P. K., Winkler, A. W. and Hoff, H. E. Electrocardiographic changes and concentration of magnesium in serum following intravenous injection of magnesium salts. Am. J. Physiol., **126:** 720–730, 1939.

(123) Smith, P. K., Winkler, A. W. and Hoff, H. E. The pharmacological actions of parenterally administered magnesium salts. A review. Anesthesiology, **3:** 323–330, 1942.

(124) Smith, P. K., Winkler, A. W. and Schwartz, B. M. The distribution of magnesium following the parenteral administration of magnesium sulfate. J. Biol. Chem., **129:** 51–56, 1939.

(125) Spier, H. W. Kombinierte gesamt-Calcium-und-Magnesium Bestimmung in Serum (Oxydimetrische Magnesiumanalyse über Mg-oxinat). Klin. Wchnschr., **29:** 85–87, 1951.

(126) Stanbury, J. B. The blocking action of magnesium ion on sympathetic ganglia. J. Pharmacol. & Exper. Therap., **93:** 52–62, 1948.

(127) Stanbury, J. B. and Farah, A. Effects of the magnesium ion on the heart and on its response to digoxin. J. Pharmacol. & Exper. Therap., **100:** 445–453, 1950.

(128) Starkenstein, E. Untersuchungen über die Magnesiumnarkose. I. Zentralbl. Physiol., **28:** 63–70, 1914.

(129) Stone, K. G. and Furman, N. H. Polarographic use of organic reagents. Magnesium with 8-hydroxyquinoline. Indust. & Engin. Chem., **16:** 596–598, 1944.

(130) Stoner, H. B. Effect of magnesium on P^{31} and P^{32} partition in muscle. Am. J. Physiol., **161:** 387–398, 1950.

(131) Stansky, E. Untersuchungen über die Magnesiumnarkose. Arch. exper. Path. u. Pharmakol., **78:** 122–153, 1915.

(132) Straub, F. B. Actin. Stud. Inst. Med. Chem. Univ. Szeged, **2:** 3–15, 1942.

(133) Straub, W. Experimentelle Untersuchung über Wesen und Aussicht der Tetanustherapie mit Magnesiumsulfat. München. med. Wchnschr., **62:** 25–29, 1915.

(134) Terkildsen, T. Chr. Electrochemical microdetermination of magnesium. Scandinav. J. Clin. & Lab. Investigation, **4:** 27–35, 1952.

(135) Tufts, E. V. and Greenberg, D. M. The biochemistry of magnesium deficiency. I. Chemical changes resulting from magnesium deprivation. J. Biol. Chem., **122:** 693–714, 1937–1938.

(136) Walaas, O. Polarographic determination of magnesium in serum by aid of 8-hydroxyquinoline. Scandinav. J. Clin. & Lab. Investigation, **1:** 187–190, 1949.

(137) Washburn, M. L. and Shear, M. J. Composition of bone. VIII. Direct gravimetric determination of Ca, Mg, and PO_4. J. Biol. Chem., **99:** 21–41, 1932–1933.

(138) Wiechmann, E. Zur Theorie der Magnesiumnarkose. Arch. f. d. ges. Physiol., **182:** 74–103, 1920.

(139) Wiki, B. Sur l'action anesthésiante locale du sulfate de magnésium. Arch. internat. de pharmacodyn. et de thérap., **21:** 415–423, 1911.

(140) Zadina, R. and Kriz, V. L'action du magnésium sur la contraction de l'intestin isolé. Compt. rend. Soc. biol., **142:** 1037–1039, 1948.

AMINE OXIDASE AND AMINE METABOLISM

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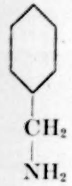
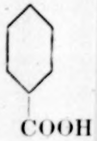
In the present review the attempt is made to correlate what is known of the metabolism of amines in the living organism with the properties and distribution of the enzyme amine oxidase. Our knowledge as to the functional significance of this enzyme is far from complete, but it seems worth while to set out what is known and what must be left to further study.

HISTORICAL

Work on the fate of amines in the living animal preceded the discovery by Miss Hare (now Mrs. Bernheim) in 1928 of an enzyme that catalyses the oxidative deamination of tyramine (86). During the nineteen thirties, there arose out of the study of the metabolism of adrenaline *in vitro* (33) and of the simple aliphatic amines (134) the concept of amine oxidase, an enzyme system which catalyses an analogous oxidation reaction of a great number of amines (112, 34, 135).

Early work on amine metabolism. The first clear indication that amines are broken down in deamination reactions came from a study of the fate of benzylamine in the organism. In 1877, Schmiedeberg (155) showed that in the dog benzylamine given by mouth was excreted as hippuric acid (benzoylglycine). He recognised that this involved the intermediate formation of benzoic acid and arrived at the generalisation that not only benzylamine,


benzylamine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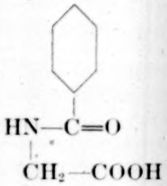
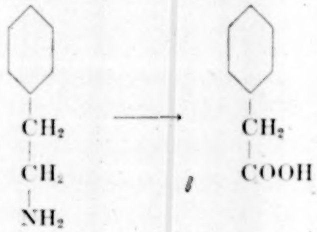

hippuric acid

but "probably all the mono-amino bases in which the nitrogen is not directly linked to a benzene ring, i.e., all bases which contain the atomic grouping $-CH_2-NH_2$ are broken down in the organism with the formation of ammonia". This statement is still valid today for very many compounds which belong to this class.

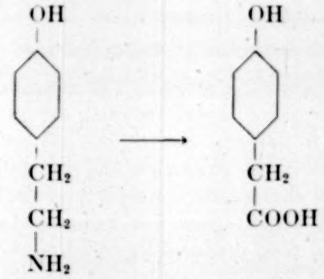
Later, both Schmiedeberg (156) and Minkowski (126) isolated free benzoic acid. Minkowski incubated minced rabbit tissues with benzylamine and found that benzoic acid was formed; thus it was shown that the deamination reaction had also occurred *in vitro*. The quantitative aspects of the metabolism of benzylamine were studied by Mosso (127); he gave the amine subcutaneously to a dog and recovered over 90% as hippuric acid in the urine.

This work on benzylamine appears to have been forgotten by the time other amines were investigated.

The next higher homologue of benzylamine, phenylethylamine, was examined by Guggenheim and Löffler (85). They gave 1 g. of phenylethylamine hydrochloride to a rabbit by mouth and recovered 0.7 g. of crude phenylacetic acid in the urine, that is, 87% of the theoretical amount.


β-phenylethylamine phenylacetic acid

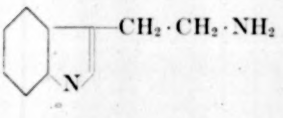
Earlier, already in 1910, Ewins and Laidlaw (65) had found that tyramine, perfused through the liver of the cat or the rabbit or through the rabbit's uterus, was destroyed and p-hydroxyphenylacetic acid was recovered, in about 70% of the maximum possible yield.



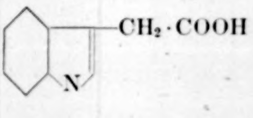
tyramine p-hydroxyphenylacetic acid

No breakdown of tyramine could be demonstrated in the perfused lung. Ewins and Laidlaw, discussing these results, wrote: "The results suggest that plain muscle having a rich sympathetic supply can effect the transformation of amine to acid; while plain muscle having a poor or no sympathetic supply is unable to do so." This connexion between adrenergic nerve supply and amine oxidase activity is a topic which will be discussed more fully below.

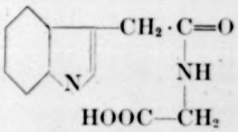
Ewins and Laidlaw also studied the fate of tryptamine (β-indolethylamine) (66). They isolated indoleacetic acid as the breakdown product of tryptamine in the perfused rabbit's liver; as the metabolic end product in the dog's urine they isolated what they thought was indoleaceturic acid, a condensation product of indoleacetic acid and glycine. Here again, deamination was the metabolic pathway.



tryptamine



indoleacetic acid



indoleaceturic acid

Similar experiments were carried out by different observers on aliphatic amines, but no characteristic breakdown products were isolated, except that with *iso*amylamine Guggenheim and Löffler (85) obtained evidence for the formation of *iso*valeric acid in the perfused rabbit's liver.

In all this older work it was assumed that the deamination of the amines was a hydrolytic reaction

$$R{-}CH_2{-}NH_2 + H_2O \longrightarrow R{-}CH_2OH + NH_3$$

i.e., a reaction in which an alcohol was formed; the alcohol was then further oxidised to the corresponding carboxylic acid. The possibility of an oxidative breakdown of the amine with formation of the aldehyde was first discussed after the discovery of "tyramine oxidase" (9); this reaction can be considered as analogous to the formation of keto acids from amino-acids as catalysed by the amino-acid oxidases (113).

PART I

Distribution of Amine Oxidase

Intracellular distribution of amine oxidase. The mammalian liver is the most convenient source of the enzyme. There are small differences in the activity and the substrate specificity in different species, but the livers of the cat, the pig, the ox, the sheep, the rabbit, the guinea-pig and the rat have been used by different authors. The most characteristic property of the enzyme is its localization in the insoluble cell constituents. For instance, the first preparations of "adrenaline oxidase" (33) were prepared by following Warburg's description of "Körnchensuspension" (177). The methods of obtaining defined fractions of the formed elements of the liver cell have since been improved and they have been applied to the study of amine oxidase. Miss Hawkins has determined the enzymic activity of various fractions of rat liver obtained when homogenates were subjected to high-speed centrifugation in isotonic and hypertonic sucrose (91). Enzymic activity was found to be present only in the particulate matter; centrifugation in 0.25M-sucrose sedimented all enzymic activity. At least 50% of the total enzymic activity was found in the mitochondria fraction. Separation of this fraction from the crude homogenate resulted in an almost threefold purification of the enzyme when activity was determined per unit of protein nitrogen. At least 25% of the total enzymic activity sedimented less readily and was found in the microsome fraction. Probably the mitochondria and microsomes account for the total amine oxidase activity of the liver homogenates (see also 48).

That amine oxidase is present in two different fractions of the particulate matter of liver cells, may explain the observation by Bernheim and Bernheim (7) that saline extracts of rabbit's liver when subjected to low-speed centrifugation will yield two fractions, one in the sediment which can be easily washed by resuspending in saline and re-centrifugation (27), and one which remains in the supernatant.

These observations make us ask: can amine oxidase be considered as a homogeneous entity? At present, it cannot be decided if the two fractions are preformed in the cell or if they arise as artefacts during the preparation of the homogenates.

Action of lytic agents on amine oxidase. Amine oxidase activity does not depend upon the structural integrity of the particles. This can be easily demonstrated when enzymic activity is measured before and after treatment of homogenates or similar preparations with agents which destroy mitochondria. Lysolecithin, a substance formed in the hydrolysis of lecithin by the lecithinase of bee or cobra venom, is a very effective lytic agent of this kind. When enough lysolecithin is added to a homogenate of guinea-pig's liver, the appearance of the homogenate changes; it becomes transparent, almost like plasma. The amine oxidase activity of a preparation thus treated remains unchanged. Amine oxidase is not the only enzyme found in mitochondria which is unaffected by treatment with lysolecithin: the rate of oxidation of indophenol before and after treatment with lysolecithin is also unchanged (16). In spite of the changed appearance of the lysolecithin-treated preparations, however, the enzymic activity is not entirely detached from particulate matter. The writer carried out an experiment with Dr. F. Himmelweit, in which a lysolecithin-treated guinea-pig liver preparation was passed through gradocol membranes; it was found that most of the activity passed through a filter of pore size 1.64 μ, but was retained on a filter of pore size 0.88 μ. The effect of lysolecithin was to disperse the large particles (mitochondria); the amine oxidase activity was now attached to particles of smaller size.

Effects similar to those seen with lysolecithin have also been obtained with bile salts. However, these substances inhibit amine oxidase, and their effect on the enzyme can only be studied after they have been removed by dialysis.

The localization of amine oxidase in the mitochondria is of interest in view of the fact that these particles contain the cytochrome system, whereas amine oxidase *in vitro* does not require the cytochrome system as a "terminal oxidase". However, we do not know if the same is true in the living cell, and the possibility that in the cell the amine oxidase reaction is linked to the cytochrome system will be discussed below. Indications for the participation of the cytochrome system may be found in experiments by Govier *et al.* (81).

In addition, the amine oxidase reaction results in the formation of two toxic metabolites, both of which are removed by reactions which take place in the mitochondria. One of these metabolites is ammonia which is removed in the mitochondria by urea synthesis, the other is an aldehyde which is further metabolised in reactions known to be linked with the cytochrome-cytochrome oxidase system. The location of amine oxidase in the mitochondria thus ensures that these two end products of the enzymic reaction are rendered harmless not far from the site where they are formed.

Amine oxidase in regenerating rat liver. Hawkins and Walker (93) have studied the regeneration of amine oxidase activity after removal of about two thirds of the liver. During the first two days after the operation a rapid increase in liver weight took place without any increase in the total amine oxidase activity. During this period, therefore, the enzymic activity per unit of weight decreased, and this decrease was made good during the following week. It is known that a lively cell division takes place on the second day after partial hepatectomy; it seems that during this period of cell division the enzyme already present is redistributed with the preformed cytoplasmatic particles (microsomes and mitochondria) over the regenerating liver cells, and the resulting "dilution" of enzyme is followed by a new formation only after the second day when the cytoplasmatic particles multiply.

This interpretation is supported by observations on hepatectomised rats which received 0.1 mg. of colchicine 24 hours after the operation. In these animals the liver weight did not change much during the 24 hours following upon the administration of colchicine. This indicates that colchicine had inhibited the new formation of liver cells. At the same time, no dilution of the enzyme occurred; the concentration of amine oxidase per unit of liver weight even rose slightly.

Distribution of amine oxidase in vertebrates. The enzyme has been found in all classes of vertebrates that have been examined (34): mammals, birds, reptiles, amphibians and teleosts. One elasmobranch species has also been examined; this is *Torpedo marmorata*, where the enzyme has been found in liver and kidneys; very little enzymic activity seems to occur in the electric organ (16).

Amine oxidase is found in many different tissues. Our knowledge of the distribution in vertebrates is mainly derived from experiments carried out on mammalian tissues. In the earlier work, enzymic activity has chiefly been studied in organs in which much enzyme is found, but interest has since shifted to tissues in which enzymic activity is relatively low. When data on amine oxidase activity obtained under different conditions are compared, it must be borne in mind that in the earlier work less efficient methods of extraction were used. Extracts were usually prepared by grinding the tissue with sand; they were then centrifuged. In this procedure an unknown amount of enzyme is lost in the sediment. Homogenates give a truer picture of total enzymic activity. The difficulties of determining total enzymic activity in tough, fibrous tissue are not yet fully overcome.

Very high enzymic activity is always found in the liver; species differences may occur, but have not been systematically studied; the guinea-pig's liver has very high enzymic activity. The kidneys also give very active preparations, but in the rat enzymic activity of the kidney is relatively low. Very active preparations can also be obtained from the pancreas and the intestine (34, 14). In sheep, the rumen, abomasum, duodenum and ileum have been examined and found to contain amine oxidase; in the duodenum and ileum, the muscular coat is richer in enzyme than is the mucous membrane (16).

In the suprarenal gland amine oxidase is present in the medulla as well as in the cortex (14, 150, 116). The enzyme occurs in other glands, *e.g.*, in the thyroid gland (14) and in the Harderian gland of the rabbit (169). It has been found in the lung (34a), including the human lung (117), but it is not known if the enzyme content of the lung is in the alveoli, in the bronchioles, or in the blood vessels. Amine oxidase has also been found in the spleen (14).

In the male sex organs, amine oxidase occurs in the testes (14), in the prostate gland and in the seminal vesicles (189). In the uterus, activity is high (14), but seems to be related to the functional state of the organ; enzymic activity is often low in the uteri of virgin rabbits (157). In a sheep which had been pregnant for 113 days, amine oxidase occurred not only in the placenta, but also in the chorion; little if any activity was present in the amnion (16). The human placenta (124) and that of other mammals (172) have been shown to have amine oxidase activity.

The heart also contains amine oxidase, but there exist marked species differences (14). Bernheim and Bernheim studied the disappearance of tyramine in the presence of slices of heart tissue from different species. They found that in some species (the rat, the rabbit and the guinea-pig) tyramine disappeared rapidly, whereas in others (cat, dog) the inactivating mechanism, probably amine oxidase, was much less potent (8). The human heart also contains amine oxidase (115).

Blood vessels have been studied by Thompson and Tickner (173); they found the enzyme in many of the blood vessels of the rabbit. The enzymic activity was high in the aorta and the renal arteries; most peripheral arteries gave preparations of lower enzymic activity. Low enzymic activity was found in veins, which confirms findings by Schapira (150). The inner half of the wall of the aorta yielded preparations as active as the outer half. In the rabbit's ear, amine oxidase has been found (150); it is present in the blood vessels of the ear (173). This is of particular interest because the observations of Gaddum and Kwiatkowski (73) on the effect of ephedrine were carried out on the blood vessels of the perfused rabbit's ear.

Skeletal muscle contains little enzymic activity, although it would seem desirable to obtain better data for this tissue with improved methods of extraction. It seems possible that the weak enzymic activity of muscle extracts can be accounted for by the enzyme present in the blood vessels contained in the tissue.

Blood is without amine oxidase activity.

Pugh and Quastel were the first to study amine oxidase in nervous tissue (134); they chiefly studied the enzyme in brain. The human brain also contains amine oxidase (15). The white matter does not contain much enzyme, and the basal ganglia are more active than the cortex. A systematic study of the enzyme in peripheral nervous tissue has not been made. In the stellate ganglion of the ox, amine oxidase has been found (183). Amine oxidase also occurs in the superior cervical and stellate ganglia of the dog; much less activity is found in the nodose ganglion (16).

The search for amine oxidase in other structures with adrenergic innervation has shown that amine oxidase occurs in the nictitating membrane and iris of cats and rabbits (145).

This list of organs and tissues with amine oxidase activity is probably not complete. It shows how widespread the enzyme is in mammalian tissues. It is difficult to make a general statement about the tissues which contain the enzyme; it is probably safe to say that it occurs in glands, plain muscle and nerve tissue.

Very little work has been done on the development of amine oxidase during growth. Epps has shown that in newborn human babies the mean enzymic activity of the kidneys—cortex as well as medulla—is about one half of the mean for adults (50; see also 190). In babies aged three months the figures for enzymic activity were as high as in adults. In the liver and the mucous membrane of the ileum Epps found no difference in the mean enzymic activity in adults and newborn babies; a surprisingly large number of extracts of duodenal mucosa from newborn babies were without any amine oxidase activity; those that were enzymatically active had a mean activity which was similar to that found in adults.

Amine oxidase in invertebrates. Only a few species representing different invertebrate phyla have been studied (34). The enzyme was not found in all phyla. No enzyme was found in annelids; this is of interest in connexion with the finding of chromaffine cells in species like the leech and the earthworm (132, 76).

Two echinoderms were examined, and both were found to contain amine oxidase; these were *Asterias rubens L.* and *Echinus esculentus L.* The enzyme was also present in the *Mollusc, Patella vulgata L.*, but not in *Helix aspersa*.

A more detailed study has since been made of the molluscan enzyme in two species of cephalopods, *Sepia officinalis* and *Octopus vulgaris*.

Sepia and *Octopus* contain in their hepatopancreas (shortly called "liver") the richest source of amine oxidase yet described (21, 30). In *Sepia*, the enzyme was present, in order of decreasing activity, in the liver, the "kidneys", the posterior salivary glands, the anterior salivary glands and ink sac; no activity was found in the musculature of the mantle. In *Octopus*, the order was: liver, posterior salivary glands, anterior salivary glands and brain. Like the mammalian enzyme, the amine oxidase of *Octopus* liver was associated with the particulate matter; after prolonged centrifugation all enzymic activity was present in the sediment.

Amine oxidase in plants and alkaloid synthesis. In earlier experiments no amine oxidase activity in plants was detected (34, 179), but more recently, Werle and Roewer (182) have prepared enzymatically active extracts from plant tissue. They demonstrated enzymic activity in the leaves, stalks and roots of *Salvia uliginosa*; in *Colchicum bornmuelleri*, in *Cannabis indica* and *Momordica balsamina*. Ammonia formation as well as oxygen consumption were measured. Like the amine oxidase of animal origin, the enzyme in plant tissue was insensitive to cyanide.

The full significance of amine oxidase in plants seems not yet to have been recognized, but it seems worth while to discuss the possibility that the enzyme has an important function in alkaloid synthesis.

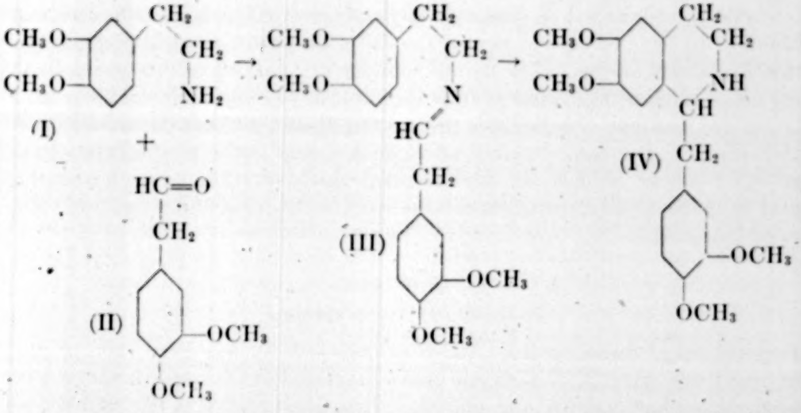
Organic chemists have for many years discussed theories of alkaloid synthesis, in order to explain the formation of the complex ring structures which are found in some of the plant alkaloids. One reaction which has been discussed is the condensation of an aldehyde with an amine:

$$R'CH_2\cdot NH_2 + R''{-}CHO \longrightarrow R'CHN{:}CHR'' + H_2O$$

(186, 146).

Reactions of this type can be studied *in vitro*, as they occur in the absence of any specific catalyst. However, catalysts must be assumed to occur which cause the amine and the aldehyde to be present at the same time. Amine oxidase is such a catalyst.

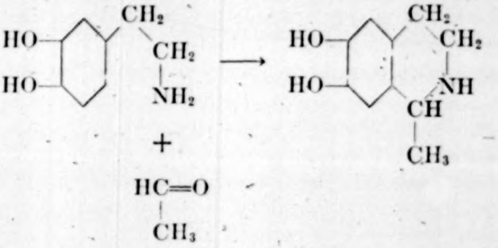
An example of such a condensation reaction which occurs *in vitro* and which may take place in alkaloid synthesis has been studied by Späth and Berger (168). In this reaction $R' = R''$. The condensation occurs between β-3:4-dimethoxyphenylethylamine (I) and the corresponding aldehyde, 3:4-dimethoxyphenylacetaldehyde (II). In the condensation reaction, the first product is believed to be compound (III) which rearranges itself to tetrahydropapaverine (IV).



The amine (I) is a substrate of the mammalian amine oxidase (14); the aldehyde (II) can therefore be produced by amine oxidase. This must mean that during the enzymic reaction both (I) and (II) are present simultaneously. It thus appears that the presence of amine oxidase creates the conditions in which alkaloid synthesis by condensation can take place.

In the living cell, condensations may also occur between amines and aldehydes in which the radicals R′ and R″ are not identical. Two condensations have been studied by Schöpf & Bayerle (158) in which the aldehyde is acetaldehyde. In the first reaction the aldehyde condenses with hydroxytyramine (V); the product isolated is 1-methyl-6:7-dihydroxy-1:2,3:4-tetrahydroisoquinoline (VI):

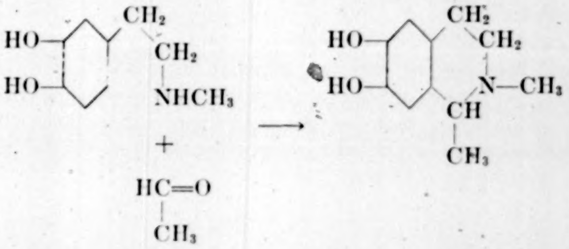
(V)



(VI)

This condensation reaction is interesting, because it is known that hydroxytyrmaine occurs in plants (154). In the second reaction acetaldehyde was incubated with epinine (VII); the reaction product was the N-methyl derivative corresponding to (VI).

(VII)



Oxidation of amines and the formation of indoleacetic acid in plants. Kenten and Mann (110) have recently extended the list of plants which yield preparations that deaminate amines. They do not consider the identity of this system with the amine oxidase of animals as fully established. Pea and lupin seedlings gave active preparations. The authors discuss the possibility that the oxidation of tryptamine to the corresponding aldehyde is a step in the biosynthesis of the growth hormone indoleacetic acid in plant tissue. For the formation of this acid in mammalian metabolism see the work of Ewins and Laidlaw (66) discussed on p. 417.

PART II

Properties of Amine Oxidase

Preparations of amine oxidase. Amine oxidase activity has often been studied in fresh tissue extracts. The enzyme used to be considered as rather labile because preparations had poor keeping qualities; it was also believed to be destroyed by organic solvents, *e.g.*, by acetone. However, it was found in 1945 that treatment with cold acetone yielded powders (*e.g.*, from mammalian liver or kidney) which contained most, if not all, the enzymic activity of the tissue. The amine oxidase activity of the acetone-dried powders is present in the insoluble residue.

This observation has corrected our views on the stability of amine oxidase: an acetone-dried powder of rabbit's liver prepared in November 1945 and kept standing in the laboratory without any precautions had still retained more than one third of its initial activity in November 1951.

The acetone-dried powder has proved a useful preparation. It is a material of known enzymic activity. It can be easily washed, a procedure which reduces the "enzyme blank". It is very suitable for the study of the reversibility of inhibitions: enzyme suspensions can be treated with a known concentration of inhibitor; reversible inhibitors can then easily be removed by washing and centrifugation (67).

Amine oxidase also withstands freeze-drying, but little work has been done with this preparation (16). In a preparation from guinea-pig liver the activity was in the insoluble material. The freeze-dried material differed in one respect from the acetone-dried powder: washing increased the enzymic activity. Possibly the treatment with acetone removes an inhibitor of the enzyme which is retained in the freeze-dried preparation.

The amine oxidase activity of *Octopus* liver is destroyed by treatment with acetone for unknown reasons; freeze-drying destroys much but not all enzymic activity. There is no indication that the loss of activity is due to the splitting off of a prosthetic group.

Homogeneity of amine oxidase. Attempts to obtain purer specimens of amine oxidase have not been carried very far, although some purification has been reported (112, 1). At present we cannot say if one and the same enzyme is responsible for the oxidation of many of the amines which have been studied. Two sets of observations can be quoted as supporting the homogeneity of amine oxidase (34):

(1) oxidation of representatives of the different types of substrates occurs in all organs in which amines are oxidised;

(2) two substrates present at the same time are not oxidised at an additive rate, but the rate of oxidation of such a mixture is intermediate between the rates of oxidation of the two amines when tested separately.

It must be borne in mind that these criteria are sometimes misleading, and it remains to be seen if the present picture will have to be modified when more purified specimens of amine oxidase become available. It is known that amine oxidases from different sources have differences in substrate specificity. This has been interpreted as an indication of the existence of different amine oxidases (1, 188). There is good evidence that a specific catalyst of the amine oxidase type with distinct properties is responsible for the oxidation of mescaline in the rabbit's liver (7, 23, 170), but even here the evidence is not entirely unambiguous. A separation of amine oxidase into two distinct enzymes has recently been reported (183); one of these is said to oxidise tyramine, the other aliphatic amines, *e.g.*, *n*-butylamine. More detailed reports of these experiments will have to be awaited before it can be said if amine oxidase is a complex which is composed of more than one enzyme.

We also do not know whether oxidation involves a sequence of reactions, *e.g.*, of hydrogen transfers interposed between substrate and molecular oxygen. It is possible that several steps are necessary, and each of these may require a specific catalyst. This will be discussed more fully below.

Oxidative deamination catalysed by amine oxidase. The reaction catalysed by "tyramine oxidase" was originally formulated thus (9):

$$(1)\qquad R\text{—}CH_2\cdot NH_2 + O_2 = R\text{—}CH{:}NH + H_2O_2$$

$$(2)\qquad R\text{—}CH{:}NH + H_2O = R\text{—}CHO + NH_3$$

In this sequence the formation of the imino compound was followed by its hydrolysis. Richter (143) replaced this formulation by equations (3) and (4) in order to allow for the fact that tertiary amines, *e.g.*, hordenine, were oxidised by the enzyme:

$$(3)\qquad R\text{—}CH_2\cdot \overset{+}{N}HR'R'' + O_2 = R\text{—}CH{:}\overset{+}{N}R'R'' + H_2O_2$$

$$(4)\qquad R\text{—}CH{:}\overset{+}{N}R'R'' + H_2O = R\text{—}CHO + \overset{+}{N}H_2R'R''$$

The overall reaction is the sum of (3) and (4):

$$(5)\quad R\text{—}CH_2\cdot \overset{+}{N}HR'R'' + O_2 = R\text{—}CHO + \overset{+}{N}H_2R'R'' + H_2O_2$$

In this formulation it is assumed that the ionised form of the amine reacts with the enzyme. We shall see that this is supported by experimental evidence: the amines which are substrates of amine oxidase are chiefly present as ions at the pH at which the enzyme is active. Amines which are not ionised to any appreciable extent are not oxidised.

Equation (5) gives a true picture when catalase is absent. In crude tissue extracts catalase is usually present, and hydrogen peroxide reacts thus:

$$(6) \qquad H_2O_2 = H_2O + \tfrac{1}{2}O_2$$

The overall equation of the enzymic reaction in the presence of catalase is thus (5) + (6):

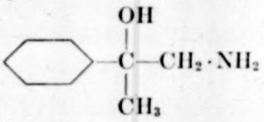
$$(7) \qquad R{-}CH_2\cdot\overset{+}{N}HR'R'' + \tfrac{1}{2}O_2 = R{-}CHO + \overset{+}{N}H_2R'R''$$

Equation (7) is reasonably well fulfilled under conditions met with in experiments with crude tissue extracts. Sometimes, however, the oxygen uptake exceeds half a molecule of oxygen for each molecule of amine added. Two factors contribute to this excessive consumption of oxygen:

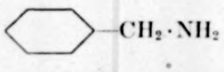
(a) some of the hydrogen peroxide formed is not decomposed according to reaction (6), but is used up in the oxidation of some organic material: such "peroxidatic" reactions are known to be catalysed by the almost ubiquitous enzyme catalase (109); the apparent increase of the rate of the amine oxidase reaction by alcohol (96) is probably explained by a peroxidatic oxidation of alcohol;

(b) the aldehyde formed is further oxidised to the corresponding carboxylic acid; such reactions are likely to occur in tissues like liver that contain an aldehyde oxidase.

Zeller (188) has recently suggested that in the histaminase reaction the primary oxidation product is: $R\text{-}CH{:}CH\cdot\overset{+}{N}HR'R''$, which rearranges itself to: $R\text{-}CH_2\cdot CH{:}\overset{+}{N}R'R''$. This interpretation is also discussed for amine oxidase; he points out that the compound



where there is no hydrogen atom in the β position, is not a substrate of amine oxidase (166). However, the fact that benzylamine



and many of its derivatives are oxidised by amine oxidase is not easily reconciled with this interpretation.

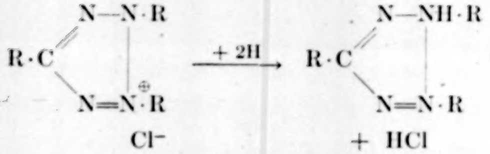
Amine oxidase and riboflavin deficiency. The mechanism of hydrogen transfer mediated by amine oxidase must remain unknown until the chemical composition of the enzyme (or of its prosthetic group) is known. The reaction catalysed is analogous to the oxidative deamination of D-amino acids by D-amino acid oxidase. Like amine oxidase, D-amino acid oxidase is cyanide-insensitive. It is a flavoprotein, and its prosthetic group is flavin-adenine-dinucleotide (FAD). In riboflavin deficiency the D-amino acid oxidase activity of rat liver extracts is known to be greatly reduced (2, 147). Miss Hawkins has recently compared the amine oxidase and D-amino acid oxidase activity of livers from riboflavin-deficient rats (92). She found that after a fortnight on the deficient diet the D-amino acid oxidase activity of liver homogenates from deficient rats had fallen to about one fifth of that from plus-riboflavin controls, whereas the amine oxidase activity was still about 50% of that of the control animals. Administration of riboflavin to the deficient animals resulted in a rapid rise of D-amino acid oxidase activity to normal values; the rise in amine oxidase activity was very small. These observations do not allow us to answer the question whether or not amine oxidase is a flavin enzyme; one is left with the impression that flavin is important, but not as directly as in D-amino acid oxidase. However, it is possible that amine oxidase is a flavin enzyme less ready to give up its flavin. Or, possibly, flavin is essential for synthesis of enzyme protein. In the presence of inositol in the diet riboflavin was more effective in restoring amine oxidase activity, but the role of inositol is not yet understood.

Amine oxidase and histaminase. Werle and Pechmann (181) believe that pyridoxal is a constituent of histaminase (see also 162). This is based on the observation that carbonyl reagents which inhibit pyridoxal enzymes, *e.g.*, the amino acid decarboxylases, also inhibit histaminase. They suggest that the histaminase reaction is initiated by a transfer of the amino groups from the diamine to pyridoxal phosphate. Pyridoxamine phosphate is then believed to be oxidised to pyridoxal phosphate by a second catalyst, possibly a flavin enzyme.

There are many similarities between histaminase and amine oxidase, but it seems very unlikely that amine oxidase contains pyridoxal. In contrast to histaminase, amine oxidase is not inhibited by carbonyl reagents, and in a few experiments on pyridoxine-deficient rats, no lowering of amine oxidase activity was observed (164).

Anaerobic reduction of dyes. In 1937, while studying the reduction of redox indicators by the amine oxidase system, Mrs. Philpot discovered the strong inhibitory action of methylene blue and toluylene blue on the enzyme (129). Two other dyes were reduced by the amine oxidase system in the Thunberg tube under anaerobic conditions; these were: o-bromophenolindophenol and o-cresolindophenol. Neither potassium indigosulphonate nor potassium indigotetrasulphonate was reduced. She thus determined the potential of the amine oxidase system as between -0.046 and $+0.195$ V.

The amine oxidase system of rabbit liver will anaerobically reduce triphenyltetrazolium chloride (TTC) (32). This substance forms a red reduction product in the presence of a fresh amine oxidase preparation and tyramine. When the rate of reduction of TTC is compared with the oxygen consumption of the same preparation under aerobic conditions, it is found that the rate of the anaerobic reaction in the presence of TTC is much slower than that of the aerobic reaction in the absence of TTC. (See 114.).



$R = C_6H_5$

In the anaerobic reduction of methylene blue by dehydrogenases, a flavin enzyme intervenes between the dye and the reduced coenzyme (pyridine nucleotide) (see 178). Similarly, for the reduction of TTC by enzymes requiring coenzyme I, a flavin enzyme is essential (37). In experiments on the amine oxidase system it has been found that the enzyme preparations lose their ability to reduce TTC more rapidly than their aerobic activity in the manometric experiment. Well washed, acetone-dried preparations will also not reduce TTC, although they are active when tested in the manometric experiment. These observations suggest that in the anaerobic reduction of TTC amine oxidase and an additional enzyme system are involved. This raises the question whether in the living organism the enzyme reacts directly with molecular oxygen or with another hydrogen acceptor which is not yet identified.

The amine oxidase system also reduces anaerobically the ditetrazolium compound described by Rutenberg *et al.* (149).

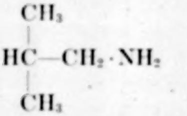
Measurement of enzymic activity. Usually the rate of the amine oxidase reaction is measured manometrically. This method is very convenient, but not very accurate; it is difficult to know if the oxygen is consumed only in the amine oxidase reaction. The measurement of the ammonia formed is more reliable (see 47), but readings cannot be taken as easily as in the manometric method. A sensitive qualitative method for the detection of the enzyme has been described by Florence and Schapira (69): tissue extracts are incubated with β-phenylethylamine in a stoppered vessel; the delicate odor of the phenylacetaldehyde formed indicates the presence of amine oxidase.

Amine oxidase and partial pressure of oxygen. The amine oxidase reaction is less rapid when the reaction is measured in an atmosphere of air instead of oxygen (129). Kohn (112) has found that in air the rate of oxidation of tyramine is about one third of that in oxygen, and in 5% oxygen it is about one third of the rate in air. The partial pressure at which amine oxidase is saturated with oxygen must therefore be very high.

This is a property in which the enzyme differs characteristically from most other respiratory enzymes which are saturated with oxygen at partial pressures which are likely to occur in the tissue. This property has been considered as an indication that the enzyme in the living tissue is very sensitive to changes of oxygen pressure (see 22). It seems more likely, however, that in the living cell amine oxidase does not transfer hydrogen to molecular oxygen, but to some other hydrogen acceptor. This hydrogen acceptor has not yet been identified.

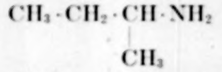
Substrate specificity. Many members of the series $CH_3(CH_2)_nNH_2$ are substrates of the enzyme (134, 112, 34, 1). Rate of oxidation and affinity vary with the number of carbon atoms in the carbon chain. The lowest homologue, methylamine, is not attacked at all. Ethylamine, however, is oxidised slowly by amine oxidase, and with increasing length of the chain the rate of oxidation increases; with 5 or 6 carbon atoms a maximum is reached and with a further increase in chain length the oxidation rate falls off. In a preparation from rabbit's liver, dodecylamine was slowly oxidised, but octadecylamine was neither oxidised nor did it act as an inhibitor of the enzyme (28). The decrease of affinity for amine oxidase with increasing chain length is almost certainly due to the low solubility of the higher members of the series and to micelle formation (111); the effective concentration of the long-chain amines is too low for oxidation to proceed at a measurable rate.

A few branched aliphatic monoamines have also been examined. Isoamylamine is rapidly oxidised. The guinea-pig liver enzyme oxidises not only n-butylamine, but also isobutylamine; sec-butylamine is not oxidised (151, 14).



$$\begin{array}{l} CH_3 \\ \;| \\ HC-CH_2\cdot NH_2 \\ \;| \\ CH_3 \end{array}$$

isobutylamine oxidised



$$\begin{array}{l} CH_3\cdot CH_2\cdot CH\cdot NH_2 \\ \qquad\qquad\quad | \\ \qquad\qquad\; CH_3 \end{array}$$

sec-butylamine not oxidised

Although the enzyme oxidises many monoamines which contain the grouping $-CH_2-NH_2$ *e.g.*, n-amylamine, it does not act on the corresponding diamines, *e.g.*, cadaverine.

oxidised by amine oxidase: $CH_3\cdot CH_2\cdot CH_2\cdot CH_2\cdot CH_2\cdot NH_2$ n-amylamine

not oxidised by amine oxidase: $NH_2\cdot CH_2\cdot CH_2\cdot CH_2\cdot CH_2\cdot CH_2\cdot NH_2$ cadaverine

The presence of the second amino group interferes with substrate specificity by lowering the affinity: putrescine and cadaverine have little or no inhibitory action on amine oxidase (18).

The short-chain diamines like putrescine and cadaverine are substrates of another enzyme, histaminase (187). This enzyme is therefore also called diamine oxidase, whereas the term "monoamine" oxidase is proposed for amine oxidase. It has been shown, however, that amine oxidase acts on a great number of diamines (28, 29), and that the lack of affinity for amine oxidase is restricted to a small number of short-chain diamines.

When the members of the homologous series of diamines $NH_2(CH_2)_nNH_2$ are examined with a preparation of amine oxidase, *e.g.*, a washed suspension of acetone-dried powder of rabbit's liver, it is found that the lower members from n = 2 to n = 6 are not oxidised, but beginning with the member with 7 methylene groups oxidation occurs; with increasing length of the polymethylene chain oxidation by amine oxidase becomes more rapid, until a maximum is reached. The compound with 13 methylene groups is most rapidly oxidised; beyond n = 13 the rate of oxidation decreases with increasing chain length (see Fig. 1).

Competition experiments have shown that not only the rate of oxidation of the diamines increases with the number of methylene groups, but also their affinity for amine oxidase: when a mixture of isoamylamine and nonamethylene diamine is oxidised by amine oxidase, the rate of oxidation is almost that with isoamylamine alone; if a mixture of the diamine with 13 $—CH_2—$ groups and isoamylamine is oxidised the rate is the same as that with the C_{13}-diamine alone.

These experiments tell us that when two basic groups are present in the molecule the second basic group in some way interferes with the attachment of the first basic group to the enzyme; this interference decreases with increasing distance between the two basic groups: the diamine with 13 methylene groups has a higher affinity for the enzyme than the monoamine isoamylamine. The gradual decrease of the oxidation rate (and affinity) with even longer polymethylene chains may be due to decreasing solubility or to micelle formation, but no information on these properties is available for the diamines.

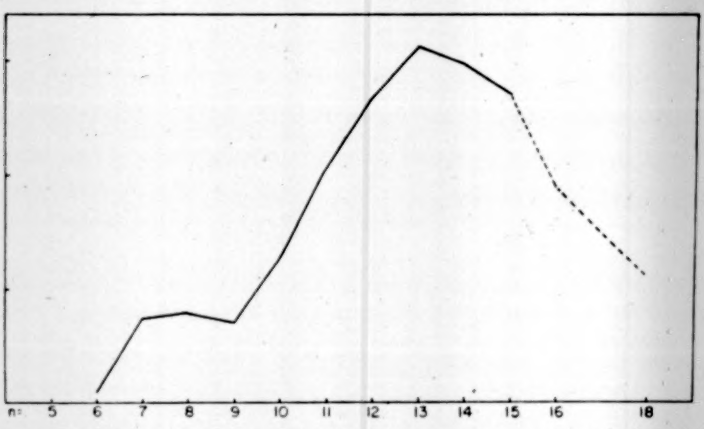


FIG. 1. Oxidation of aliphatic diamines of the homologous series $H_2N(CH_2)_nNH_2$ by amine oxidase (rabbit's liver). Abscissa: number (n) of carbon atoms in polymethylene chain; Ordinate: rate of oxidation (in arbitrary units).

The reason for the disturbing influence of the second basic group is not known. It is unlikely that it is due to changes in the dissociation constants, as it seems that there is little difference between the constants of diamines which are not oxidised and those which are (160).

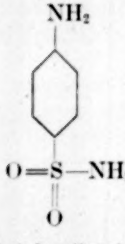
In order to understand the different behaviour of short-chain and long-chain diamines, we have to consider their orientation on the active surface of the enzyme. We have seen that in the series of aliphatic monoamines the lowest members are relatively poor substrates of amine oxidase; an optimum configuration is reached with amylamine (C_5) or hexylamine (C_6). This suggests that the presence of the group $-CH_2 \cdot NH_2$ at which the reaction occurs is not a sufficient condition for affinity; the hydrocarbon moiety is also essential. This may ensure that the substrate molecule is lifted up and arranged at an angle to the active surface (28). Short-chain diamines cannot take up such a position; they would tend to arrange themselves parallel to the surface. With increasing number of methylene groups, the orientation of the diamines becomes more like that of the monoamines and a successful enzyme-substrate reaction can occur.

Aromatic amines, like aniline, in which the amino group is directly attached to the ring, are not attacked by amine oxidase (34). If it is true that amine oxidase acts on the ionised amines, then it can easily be understood why aromatic amines are not oxidized: at pH 7.4 about 99.7% of ethylamine is present in the ionized form, but only about 0.4% of the aniline is present as ions.

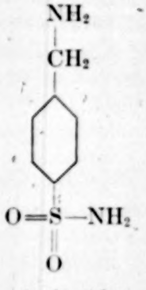
Whereas aniline and similar compounds are weak bases, the strongly basic character is re-established in aromatic compounds in which the amino group is not directly attached to the ring. It is not surprising, therefore, to find that they are substrates of amine oxidase, like the aliphatic amines.

Benzylamine, the compound studied by Schmiedeberg and his colleagues, is oxidized by amine oxidase (34). There are species differences in the rate of oxidation of benzylamine: it is more rapidly oxidized in the rabbit liver than in the guinea-pig liver (16).

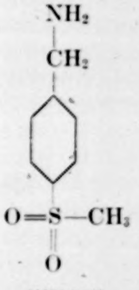
One derivative of benzylamine is of therapeutic interest; this is ambacide (Marfanil), the p-sulphamido derivative. This compound is a substrate of amine oxidase (11, 27). As would be expected, sulphanilamide is not a substrate of the enzyme; the amino group in sulphanilamide is directly attached to the ring and is not strongly ionized at pH 7.4.



Sulphanilamide



Ambacide



"V335"

The compound V335 is a substrate of amine oxidase (27).

With increasing length of the side chain the rate of oxidation increases: β-phenylethylamine is oxidised more rapidly than benzylamine and γ-phenylpropylamine is more rapidly oxidised than β-phenylethylamine (12). Amines with longer side chains appear not to have been examined.

The derivatives of β-phenylethylamine have been most carefully studied: this is because this group includes so many "biogenic" amines, *e.g.*, tyramine, adrenaline, noradrenaline, hydroxytyramine, hordenine and mescaline; in addition, many synthetic derivatives of β-phenylethylamine have therapeutic interest.

This is not the place to discuss the pharmacological properties of this group of amines. Since the classical work of Barger and Dale (4) they have been most extensively studied (see 35). The metabolism of derivatives of phenylethylamine has also been reviewed in recent years (90, 84, 10, 12), and I shall in the following discuss chiefly those aspects which are connected with the question of the activity of amine oxidase in the living organism.

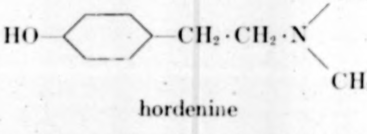
Amine oxidase was first described as "tyramine oxidase" (86), and tyramine is a typical substrate of the enzyme. Not only compounds with the phenolic group in para-position, as in tyramine, but also meta-phenolic amines like neosynephrine are oxidised (34); both the meta- and ortho-analogues of tyramine are substrates of amine oxidase (136).

Of the diphenolic derivatives of phenylethylamine the catechol amines have been most extensively studied. The biogenic amines which belong to the group are adrenaline, noradrenaline and hydroxytyramine; they are oxidised by amine oxidase and so are many synthetic catechol amines. The resorcinol derivative, 3:5-dihydroxyphenylethylamine, is also oxidised by amine oxidase (83). One derivative of hydroquinone has been studied; this is 2:5-dihydroxyphenylethylamine; although the compound was not very stable in aqueous solution, it appears to be oxidised by amine oxidase (31).

Substitution of the amino group. If one of the two hydrogens of an amino group is substituted by a methyl group, the rate of oxidation by amine oxidase is not very much affected. If the hydrogen is replaced by a more bulky group (*e.g.*, ethyl or iso-propyl) the oxidation rate is much more reduced.

If both hydrogen atoms in an amino group are replaced by methyl, the rate of oxidation is very much reduced. Other tertiary amines like tri-butylamine or tri-isoamylamine are not oxidised at a measurable rate (34; see also 1).

A tertiary amine which was shown to be oxidised by amine oxidase is hordenine, the N-dimethyl derivative of tyramine (112):



hordenine

The rate of oxidation of hordenine differs in different species: in the guinea-pig's liver it is much more slowly oxidized than in the pig's kidney (34). That the difference in the oxidation rate of tertiary amines is a species difference was confirmed by Randall (136) in a systematic study of the effect of progressive N-methylation on the rate of oxidation by amine oxidase. He examined a great number of phenylethylamine derivatives, always comparing the rate of oxidation of the primary, the secondary and the tertiary amine. Two enzyme preparations were used, one from guinea-pig's liver, the other from cat's liver. He did not find marked differences in the rates of oxidation of the primary and secondary amines; sometimes the primary, sometimes the secondary amine was attacked a little more readily. There was a systematic difference in the rate of oxidation of the tertiary amines by the liver preparations of the two species: the rate of oxidation was always less than with the primary and secondary amines, but this reduction was much greater with the guinea-pig liver enzyme than with the cat liver enzyme.

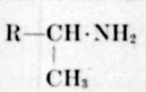
Quaternary ammonium compounds are not oxidised by amine oxidase.

Adrenaline differs from noradrenaline by one N-methyl group. Both amines are oxidized by amine oxidase (34), and the rate of oxidation is similar. When an equimolecular mixture of the two amines is oxidized by an acetone-dried powder of rabbit's liver, the ratio: $\frac{\text{adrenaline}}{\text{noradrenaline}}$ increases as the reaction proceeds. In other words, in the mixture noradrenaline is more rapidly oxidised (25, 42). The affinity of noradrenaline for amine oxidase has not been determined. Figures for the affinity of adrenaline for the enzyme have been given (112, 34), but it should be remembered that the measurement of the oxidation rates of the catechol amines are made inaccurate by the fact that some oxygen is consumed in reactions other than that catalysed by amine oxidase.

The side chain hydroxyl group. Noradrenaline and adrenaline both contain the hydroxyl group on the carbon atom adjacent to the carbon atom which carries the amino or methyl-amino group. The presence of this hydroxyl group lowers the affinity of the amines for amine oxidase. It is probably due to this fact that the oxidation of adrenaline by amine oxidase was not at first noticed and only described in 1937 (33).

Competitive inhibitors of amine oxidase. It has already been mentioned that compounds like tri-isoamylamine, in which the nitrogen atom carries more than one aliphatic substituent, are not oxidized by amine oxidase. They inhibit the enzyme; this type of inhibition may well be a competitive one (see 1). A sharp line between competitive inhibitors and substrates cannot always be drawn. Any substrate of amine oxidase, because it has affinity for the enzyme, may interfere with the oxidation of another substrate. This is the basis of the phenomenon of substrate competition. If the affinity of a given substrate for amine oxidase is high, but its rate of oxidation slow, it will act as an inhibitor. A well-known example of this kind is the inhibition of the cholinesterase of serum by eserine, which is a substrate with a high affinity for the enzyme, but which is hydrolysed at a very slow rate (49).

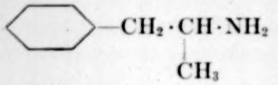
There is one group of amines which are not oxidised by the enzyme at a measurable rate, but which act as competitive inhibitors of amine oxidase: these are compounds in which the amino group is not attached to a terminal carbon atom. Most of the substances studied have a methyl group attached to the carbon atom which is immediately adjacent to the basic group:

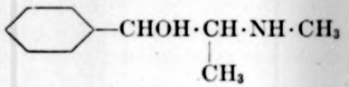


$$R\text{—}\underset{\displaystyle CH_3}{\underset{|}{CH}}\cdot NH_2$$

Ephedrine was the first inhibitor of this group which was studied, but it was soon found that this property was shared by many other derivatives of β-phenylisopropylamine (17) and by β-phenylisopropylamine itself (125).

When the percentage inhibitions caused by equimolar concentrations of phenylisopropylamine derivatives are determined, it is found that these substances fall into two classes of different inhibitory strengths (20). The class which includes phenylisopropylamine itself contains more potent inhibitors than the class which includes ephedrine.

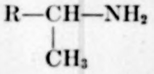

β-phenylisopropylamine
(amphetamine)


ephedrine

The second class differs from the first in the presence of the hydroxyl group in the side chain. This shows that the hydroxyl group in the side chain affects the affinity of competitive inhibitors in the same way as that of substrates, *e.g.*, of the adrenaline-noradrenaline type.

The competitive character of the amphetamine inhibition was studied by Mann and Quastel (125) who found that the competition of amphetamine with tyramine for amine oxidase was strictly reversible. They confirmed the weaker inhibitory action of compounds of the ephedrine type.

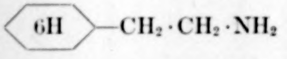
The inhibitory action of amines of the general structure

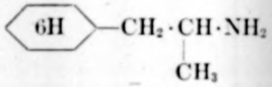


$$R—\underset{\displaystyle CH_3}{\underset{|}{CH}}—NH_2$$

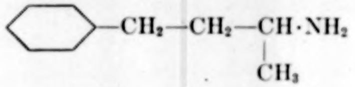
has often been confirmed (128, 94, 95, 68). Heegaard and Alles have shown that also in the aliphatic series the amines in which the amino group is not attached to a terminal carbon atom act as inhibitors.

Similarly, β-cyclohexylethylamine is a substrate of amine oxidase, but β-cyclohexylisopropylamine is not oxidised (167):


oxidised


not oxidised

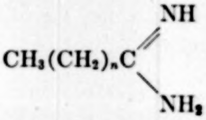
Fellows and Bernheim have recently shown that 2-amino-4-phenyl-n-butane and some of its derivatives also inhibit amine oxidase (68).



Amidines as inhibitors of amine oxidase. Many amidines are inhibitors of amine oxidase (27, 67; see also 6). It is likely that these compounds inhibit the enzyme because their basic group combines with the receptor for the amino group.

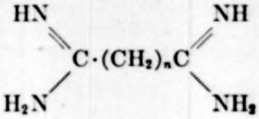
A study of the relation between the chemical constitution of amidine compounds and inhibitory strength has been made in several homologous series of amidine derivatives. These include:

(1) Monoamidines:



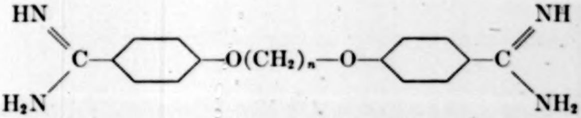
$$\mathrm{CH_3(CH_2)_nC}\begin{matrix}\diagup\!\!\diagup\mathrm{NH}\\ \diagdown\mathrm{NH_2}\end{matrix}$$

(2) Diamidines:

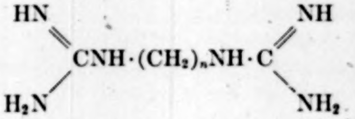


$$\begin{matrix}\mathrm{HN}\diagdown\!\!\diagdown\\ \mathrm{H_2N}\diagup\end{matrix}\mathrm{C\cdot(CH_2)_nC}\begin{matrix}\diagup\!\!\diagup\mathrm{NH}\\ \diagdown\mathrm{NH_2}\end{matrix}$$

(3) Propamidine-
Pentamidine
series:

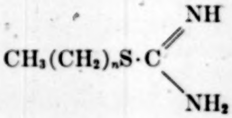


(4) Diguanidines:



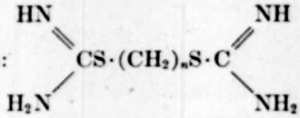
$$\begin{matrix}\mathrm{HN}\diagdown\!\!\diagdown\\ \mathrm{H_2N}\diagup\end{matrix}\mathrm{CNH\cdot(CH_2)_nNH\cdot C}\begin{matrix}\diagup\!\!\diagup\mathrm{NH}\\ \diagdown\mathrm{NH_2}\end{matrix}$$

(5) Mono*iso*thioureas:



$$\mathrm{CH_3(CH_2)_nS\cdot C}\begin{matrix}\diagup\!\!\diagup\mathrm{NH}\\ \diagdown\mathrm{NH_2}\end{matrix}$$

(6) Di*iso*thioureas:

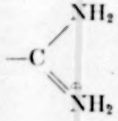


$$\begin{matrix}\mathrm{HN}\diagdown\!\!\diagdown\\ \mathrm{H_2N}\diagup\end{matrix}\mathrm{CS\cdot(CH_2)_nS\cdot C}\begin{matrix}\diagup\!\!\diagup\mathrm{NH}\\ \diagdown\mathrm{NH_2}\end{matrix}$$

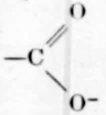
Certain general rules emerge from the study of the inhibitory action of these compounds. Firstly, in the monoamidine series ((1) and (5)), the first members have little inhibitory action; the inhibition rises at first with increasing chain length, but falls off again after a maximum is reached. Secondly, the series of diamidines ((2), (3), (4) and (6)) contain stronger inhibitors of amine oxidase than the mono-substituted compounds; propamidine and pentamidine are the most active inhibitors of amine oxidase so far described. Thirdly, in the di-substituted series the maximum inhibitions are reached with longer chains than in the mono-substituted series.

It can be seen that the affinity of amine oxidase for the amidine compounds is analogous in many ways, to the affinity of the enzyme for the amines, except that the latter are oxidised whereas the former inhibit. In the monoamine series the short-chain compounds are slowly oxidised and, after a maximum is reached, the rate of oxidation and affinity fall off with increasing chain length, and in the diamine series, the long-chain members are oxidised with an optimum chain length which is greater than in the monoamine series.

These analogies suggest that the amidine group combines with the active centre in the enzyme which normally reacts with the amino group. The amidines are even stronger bases than the amines and they are fully ionised at the pH of physiological interest. It has been suggested by Walker (176) that the ionised amidinium group:



reacts with the ionised carboxyl group:



to form a ring which is stabilised by resonance. Possibly the enzyme protein reacts with the amidines in a similar way.

Pentamidine and some other amidine compounds are very firmly held by the enzyme and not easily removed by washing (27, 67). A more detailed study of the reversibility of the inhibition has been made for some of the mono*iso*thioureas (series (5)); in this series the removal of the inhibitor by washing becomes less complete as chain length increases. The n-butyl derivative was completely removed after three washings; the n-hexyl derivative was almost fully removed after six washings; the n-octyl derivative was incompletely removed after six washings, and there was very little indication of removal of the n-decyl derivative after six washings. In these experiments the inhibitor concentrations were chosen so as to produce about equal percentage inhibitions of the enzyme (about 60–80%); the concentrations were:

of S-*n*-butyl *iso*thiourea: 25.0×10^{-3} M
of S-*n*-hexyl *iso*thiourea: 6×10^{-3} M
of S-*n*-octyl *iso*thiourea: 1.2×10^{-3} M
of S-*n*-decyl *iso*thiourea: 0.2×10^{-3} M

The most firmly held compound is the strongest inhibitor; the feeble inhibitor is easily removed by washing.

These observations suggest that the reaction between amidine group and active group of the enzyme does not determine the force by which the amidine is held on the enzyme, but this depends upon the nonspecific forces between the non-polar moiety of the amidine molecule and the enzyme surface. The reaction between amidines and enzyme can thus be considered as a model of the reaction between the enzyme and amines.

Other inhibitors

Methylene blue. The strong inhibitory action of methylene blue has already been mentioned. Not all redox indicators are inhibitors of the enzyme (129, 131).

The mechanism of the inhibition by methylene blue is not known. Although it is a strong inhibitor of the enzyme, this seems to depend upon experimental conditions. Some authors report that, with low partial pressures of oxygen, methylene blue causes an increase of the rate of oxygen uptake (112, 81).

Sulphydryl reagents. Amine oxidase is inhibited by —SH reagents (70, 163). The concentration of inhibitor must be fairly high. The inhibition is partly reversed by glutathione.

Metal-complex formers. Cyanide, carbon monoxide and azide are not inhibitors of the enzyme, and there is at present no evidence that a heavy metal is part of amine oxidase.

Carbonyl reagents do not inhibit the enzyme. Semicarbazide is often used to ensure that the aldehyde formed in the enzymic reaction is not further oxidised, *e.g.*, by aldehyde oxidase; it is without any action upon the oxygen uptake due to amine oxidase.

Alcohols. Octyl alcohol is an inhibitor of the enzyme. Heim (96) has recently studied the action of the homologous series of aliphatic alcohols on the enzyme: the lower members increase the oxygen consumption, but the higher members inhibit. This increase of the oxygen uptake is probably not due to any activating effect of the lower alcohols on the enzyme, but to the oxidation of the alcohols by the hydrogen peroxide formed.

The action of local anaesthetics. The inhibitory action of cocaine and many related substances on amine oxidase was first described by Mrs. Philpot (130); this effect has often been confirmed (128, 97, 98). Percaine is a very strong inhibitor of the amine oxidase from guinea-pig liver; then there follow in decreasing order of inhibitory activity, cocaine, stovaine, butyn and procaine (128). In liver extracts, some anaesthetics which contain ester linkages are hydrolysed by enzymes present in the tissue; they lose their inhibitory action as hydrolysis proceeds (98). In kidney extracts, where esterases are absent, no hydrolysis occurs and the inhibitory action on amine oxidase is retained.

Antihistamines. Schuler (159) showed that antistin was an inhibitor of amine oxidase. This was confirmed by Tickner (174) who found that not only antistin but a number of other antihistaminics were inhibitors of amine oxidase. He used a rabbit's liver homogenate as source of enzyme and tyramine in a concentration of about 10^{-3} M as substrate. Under these conditions 10^{-3} M diphenhydramine hydrochloride (benadryl) caused an inhibition of about 50%; the weakest inhibitor was mepyramine with 32% inhibition.

The sympathetic blocking agents 933F (174), ergotoxine (16), ergotamine and ergometrine (128), also are weak inhibitors of amine oxidase.

An inhibition of the enzyme by xanthine derivatives, *e.g.*, by theophylline, caffeine and theobromine, has also been described (99).

PART III

Amine Oxidase and Amine Metabolism in the Living Organism

It is often difficult to derive a clear picture of the mode of action of an enzyme in the living animal from the study of its properties *in vitro*. This is true also for amine oxidase. Two main questions which we are unable at present to answer may be briefly stated at the outset:

(a) We do not know if in the living cell amine oxidase reacts directly with molecular oxygen as it does in the manometer vessel or whether a chain of oxidoreduction catalysts take part in the reaction. I have already discussed some observations which suggest that the enzyme does not normally react with molecular oxygen. If this is so, then measurements of enzymic activity made so far do not represent the true rate of the enzymic reaction *in vivo*. Data obtained in manometric experiments give relative rates only of enzyme activity, but the rate at which amines are broken down in the tissues may be different.

(b) We do not know if in the body the deamination of amines is the only reaction catalysed by the enzyme; it seems possible that in the organism the amine oxidase reaction, coupled with an energy-yielding reaction, runs in the reverse direction, *i.e.*, in the direction of amine formation. Here we have no experimental data which can help us. We can only say that we do not know of any reactions in which the aldehydes could be formed which might be the precursors of the biogenic amines found in the animal body.

In the following, I shall discuss the role of amine oxidase, first in the metabolism of substances which are not normally found in the body, and secondly, in the metabolism of amines which might normally be substrates of the enzyme

A. Metabolism of amines not normally found in the body

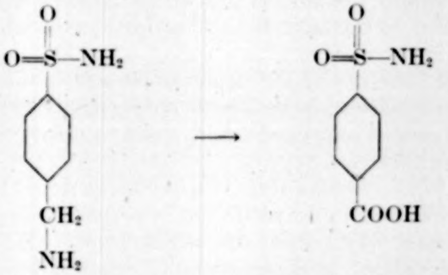
The fate of the simple aliphatic monoamines is breakdown by deamination. No characteristic products of the metabolism of straight-chain monoamines have been described, but it is known that they increase the output of urinary urea (122). We can ask: is there an agreement between the deamination of these substances *in vivo* and their oxidation by amine oxidase?

Methylamine, the simplest of the aliphatic amines, presents an anomaly. In the living organism, it is broken down completely (142) and its nitrogen is excreted as urea. There is no evidence to suggest that amine oxidase acts on methylamine. We must conclude that amine oxidase is not the enzyme responsible for its breakdown.

In man, dimethylamine is not metabolised, but recovered in almost theoretical amounts from the urine (142). Dimethylamine is also not oxidized by amine oxidase.

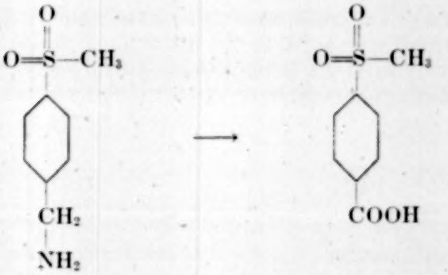
The excretion of some other aliphatic amines in man has been studied by Rechenberger (142). He found that 32% of ethylamine was recovered from urine, but only about 10% of propylamine and 2% of *n*-butylamine. Of diethylamine, 86% was recovered from urine, and about 15% of *iso*butylamine. These data are easily interpreted on the assumption that amine oxidase is the catalyst responsible for deamination in the living organism of aliphatic monoamines. We have seen that for the short-chain monoamines the rate of oxidation by amine oxidase rises with increasing chain length. It is therefore not difficult to understand that about one third of the ethylamine escapes destruction, whereas of propylamine more is destroyed and of *n*-butylamine even more. *Iso*butylamine is oxidised much less rapidly by amine oxidase than *n*-butylamine (142), and diethylamine is scarcely oxidised by the enzyme. In the dog, 1 g. doses of propylamine, given on 10 consecutive days, completely disappeared (5). *Iso*amylamine, a good substrate of amine oxidase, is also completely metabolised in the dog; in the perfused liver, the metabolic product isolated is *iso*valeric acid (122). This is the product we would expect to find if amine oxidase was active; the intermediate product, *iso*valeraldehyde, would be expected to be oxidised by the aldehyde oxidase of liver to the corresponding carboxylic acid.

Benzylamine and benzylamine derivatives are excreted as the corresponding carboxylic acids. We have already discussed the work of Schmiedeberg and his school on benzylamine which is excreted as benzoic acid (or hippuric acid); it is interesting that this amine is fully metabolised as we would expect it to be if amine oxidase were the catalyst of the primary breakdown reaction. Similarly, 84% of the theoretical amount of ambacide appears in rabbits urine as p-carboxybenzenesulphonamide, and 87% of "V 335" appears as p-methylsulphonylbenzoic acid (88):



Ambacide

p-carboxybenzene-
sulphonamide



V 335

p-methylsulphonyl-
benzoic acid

Hartles and Williams have also studied the fate of ingested p-hydroxybenzylamine in the rabbit; 40% was excreted as free p-hydroxybenzoic acid, 39% as the glucuronide of p-hydroxybenzoic acid and 19% as its sulphate (89). Here again, it seems probable that amine oxidase was the catalyst; it is not known if oxidation to the free hydroxy acid preceded esterification or if the esterified amines were oxidised by amine oxidase.

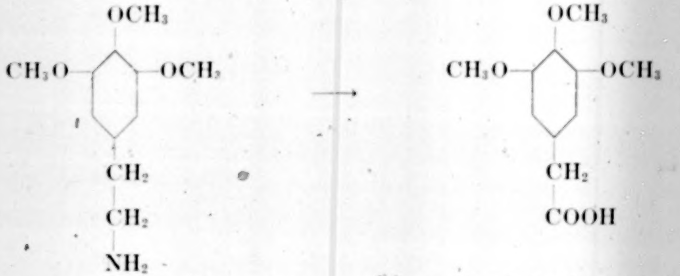
It seems particularly interesting that the benzylamine derivatives are converted to the carboxylic acid so quantitatively. Like benzylamine itself, they are not very rapidly attacked by amine oxidase. We can therefore conclude that in the living body the enzyme is able to attack amines which are only slowly oxidised *in vitro*.

The fate of phenylethylamine and of tyramine has already been mentioned in the introduction when the work of Ewins and Laidlaw and of Guggenheim and Löffler was discussed (65, 85). Here again, the carboxylic acids are recovered; this indicates that amine oxidase has been the catalyst. There is one phenolic amine which is an exception to the general rule: 2:5-dihydroxyphenylethylamine is oxidized in the body without giving rise to characteristic breakdown products (120). This can be easily understood: amine oxidase would be expected to oxidize the amine to the corresponding aldehyde (31); the aldehyde would be further oxidized to the corresponding carboxylic acid, homogentisic acid. The metabolic pathway would from then on be the same as for tyrosine which is also broken down via homogentisic acid and fully oxidised.

Conversely, many amines which are not attacked by amine oxidase are excreted unchanged. This is true for amphetamine and ephedrine (144). The resistance to amine oxidase is responsible for the characteristic long-lasting effect of ephedrine and related compounds which was first clearly recognised by Chen and his colleagues (45).

Amphetamine is not quantitatively excreted in the urine (105, 105a, 118, 13). Some of it is metabolised. In the rabbit this breakdown of amphetamine is more marked than in man, where about one half is excreted (87). This inactivation may also be by oxidation, but not catalysed by amine oxidase; it is possible that ascorbic acid acts as hydrogen acceptor in a dehydrogenation reaction which precedes deamination (10).

The metabolism of mescaline presents a special case. In man, about 50% of the amine is excreted unchanged in the urine (144), but in the rabbit it is almost quantitatively excreted as the corresponding carboxylic acid, 3, 4, 5-trimethoxyphenylacetic acid (165):



Mescaline 3, 4, 5-trimethoxyphenylacetic acid

That mescaline is destroyed in the rabbit is in agreement with observations on tissue preparations: the liver of the rabbit differs from that of most other mammals in that it contains an enzyme that actively deaminates mescaline (7).

We can sum up the results of all these observations by saying that catalysts of the amine oxidase type must be assumed to be the chief means of inactivation of amines in the organism, but that in special instances other mechanisms are at work. It seems remarkable that even substances which are very slowly oxidised *in vitro* are metabolised in the living animal as one would expect them to be metabolised if amine oxidase were the catalyst responsible for the first step of oxidation.

B. The biological significance of amine oxidase

As more is learned about "detoxication reactions", it becomes evident that they represent metabolic pathways of chemically related substances which normally occur in the body. These normal metabolites are usually broken down completely, often to water, carbon dioxide and ammonia (or urea), end products which do not tell us much about the metabolic pathways followed. Until the introduction of isotopic tracer techniques, most of our knowledge of these metabolic pathways came from the study of foreign substances which were not broken down completely, but which led to characteristic end products that gave some information about the steps by which the breakdown had occurred.

We have to ask: is amine oxidase an enzyme which catalyses the breakdown of substances normally found in the body or does it chiefly deal with substances introduced from outside?

Amine oxidase as "detoxicating" agent. It seems quite possible that amine oxidase does serve some truly "detoxicating" functions. As has been pointed out some time ago, the high concentration of the enzyme in liver and intestine may prevent amines taken up from the alimentary canal from reaching the general circulation (14). But we have to ask if the uptake of amines, such as tyramine, from the intestine is an event that is likely to occur on a large scale and often enough to require the presence of a specific catalyst in so many different tissues.

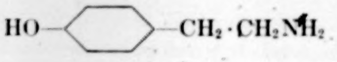
Bacterial formation of amines takes place in an acid milieu and is completely suppressed in an alkaline environment. The enzymic formation of tyramine from tyrosine by *Streptococcus faecalis* is at an optimum at pH 5.0–5.5 (75). Moreover, the bacterial amino-acid decarboxylases are adaptive enzymes which are formed in the bacterial cell only when growth occurs in an acid environment (74). These are conditions not very often associated in the intestinal lumen with a high bacterial activity. Thus it seems that only under exceptional conditions would amine oxidase be necessary to destroy amines formed in the gut.

It may, however, happen that the food has been exposed to putrefaction and that the amines are already present in it when it is eaten. This may occur more often in animals which normally feed on putrefying meat. It would be interesting to know if the tissues of animals with such feeding habits are particularly rich in amine oxidase.

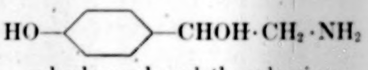
Amine oxidase is present in so many tissues other than liver and intestine that it seems unlikely that the enzyme serves only the function of removing amines taken up from the intestine. We have to enquire if amines which can be substrates of amine oxidase *in vivo* are normally found in the body.

Sympathicomimetic amines

Invertebrates. Tyramine was first found by Henze (100) in the posterior salivary glands of *Octopus macropus*. This has since been confirmed by Erspamer who found tyramine also in the posterior salivary glands of *Octopus vulgaris*, a closely related species, where it occurs together with another monophenolic amine, octopamine (56). From a study of extracts of posterior salivary glands of *O. vulgaris* by paper chromatography it seems likely that octopamine is l-p-hydroxyphenylethanolamine (60, 58), an amine closely related to tyramine:



tyramine



p-hydroxyphenylethanolamine

Final proof of the identity of octopamine with hydroxyphenylethanolamine will have to await isolation and chemical identification of the amine.

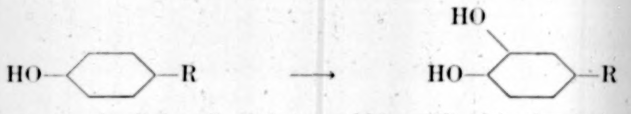
The function of these amines in *Octopods* is not yet known. Originally it was believed that tyramine was the poisonous principle of the saliva, but this is contested. Sereni (161) believed that tyramine acted as a hormone in these animals.

The tissues of *Octopus vulgaris* are rich in amine oxidase. The posterior salivary glands where these amines are found is second only to the "liver" in enzymic activity (30). The *Octopus* enzyme, like the mammalian enzyme, oxidizes both tyramine and p-hydroxyphenylethanolamine (16). Data summarized in the following table will show that the rate of oxidation of the ethanolamine derivative is much slower than that of tyramine. (In the table, the initial oxygen uptake is expressed in μl. O_2 consumed per 100 mg. of fresh weight per hour.)

TISSUE	WITH TYRAMINE	WITH dl-p-HYDROXYPHENYLETHANOLAMINE	WITH BOTH AMINES
rabbit liver	200	38	182
Octopus "liver"	254	74	198
Octopus posterior salivary glands	44	8.5	26.5

The slow oxidation of the ethanolamine derivative is in agreement with what is known about the introduction of a hydroxyl group in β-position to the amino group.

It has recently been found by Ghiretti that the tyrosinase of *Cephalopods* is able to oxidize tyramine (78); this suggests that these animals may also form hydroxytyramine (from tyramine) and noradrenaline (from "octopamine"). The diphenolic amines would be the primary oxidation products of the corresponding monophenolic amines in the tyrosinase reaction.



In tissue extracts the diphenolic amines would be oxidised further to compounds of the adrenochrome type.

The occurrence of p-hydroxyphenylethanolamine is also of interest in connexion with the problem of biosynthesis of adrenaline and sympathin; possible pathways involving hydroxyphenylethanolamine have been discussed elsewhere (26).

The presence in *Octopods* of both sympathicomimetic amines and amine oxidase makes it possible that the function of the enzyme is to be found in the removal of these amines. It would clearly be desirable to have more precise information about the part played by these amines in *Octopods* before the significance of amine oxidase can be discussed. If they act as hormones, the enzyme may well serve a local inactivation mechanism. As in vertebrates, the chief digestive gland ("liver") is very rich in enzyme; this may be useful in the "detoxication" of amines taken up in the food. It seems difficult to believe that the enzyme serves a similar purpose in the other organs in which it has been found.

Other invertebrate phyla have not been nearly as fully studied. In echinoderms, where amine oxidase has been found (34), nothing is known about the occurrence of sympathicomimetic amines. On the other hand, annelids like the leech and the earthworm contain adrenaline (77) and probably also noradrenaline (62), but no amine oxidase has hitherto been found in these species. It must be admitted, however, that the method of demonstrating the presence of the enzyme was very crude: the entire animal was ground up in a mortar and examined; since it is known that only a few structures in the vascular and digestive system have adrenergic innervation, it would be interesting to examine these for the presence of the enzyme.

Vertebrates. The occurrence of sympathicomimetic amines in vertebrates has recently been discussed in these Reviews by Euler (63). Three sympathicomimetic amines have so far been found: adrenaline, noradrenaline and hydroxytyramine.* Only the first two are known to act as hormone and mediator; for hydroxytyramine no similar function has hitherto been established. Both hydroxytyramine and noradrenaline are associated with adrenaline not only in nervous and endocrine tissue, but also in exocrine tissue: noradrenaline occurs in small amounts in the secretion of the Chinese toad (*Bufo gargarizans*) (121), and hydroxytyramine occurs in the parotoid gland of *Bufo marinus* (82). In mammals, hydroxytyramine has been found in the heart and the adrenal medulla (80).

That the three amines are so often found together supports the idea of a genetic relationship: hydroxytyramine may be considered as a precursor of noradrenaline, and noradrenaline in its turn as a precursor of adrenaline (24). This does not mean that the three amines do not have their own specific regulatory functions.

Hydroxytyramine is formed by the enzyme DOPA decarboxylase which was discovered by Holtz, Heise and Ludtke in the mammalian kidney (103), but it has since been found in the liver (19), in the pancreas (102), in the guinea-pig's intestine (101) and in the adrenal medulla of the ox (116).

Hydroxytyramine is rapidly oxidised by amine oxidase (34), and its affinity for amine oxidase is high (1, 10). It is therefore to be considered as an important substrate of amine oxidase in the living animal. It seems doubtful if hydroxytyramine is quantitatively transformed to noradrenaline.

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* The term "dopamine" has recently been proposed by Dale for what is here called hydroxytyramine; this new name is preferable because it is less ambiguous and because it stresses the relationship between amino-acid and amine.

The finding of DOPA decarboxylase in the suprarenal medulla of the ox makes it likely that, at least in this species, hydroxytyramine is formed for the production of adrenaline and noradrenaline in the gland. It is not known whether this is the same in other species; the large amounts of enzyme, especially in liver and kidneys, may not only serve the function of producing hydroxytyramine for synthesis of noradrenaline and adrenaline, but we must consider the possibility that hydroxytyramine itself has some important regulatory function which is not yet recognized. If this is so, amine oxidase may be a mechanism which removes surplus hydroxytyramine not required for conversion into noradrenaline or adrenaline.

Amine oxidase occurs wherever the DOPA decarboxylase has been found, but the oxidase appears to have a much wider distribution. This may indicate that amine oxidase acts also in tissues other than those in which hydroxytyramine is formed, but possibly DOPA decarboxylase is more widely distributed than is known at present. The decarboxylase is a pyridoxalphosphate enzyme, and it may be that small amounts escape detection in tissue extracts in the absence of added codecarboxylase, as it is known that the enzyme tends to become dissociated when it is highly diluted.

A functional relationship between DOPA decarboxylase and amine oxidase is also discussed by Polonovski and his colleagues (150, 133, 79). This is based on an earlier observation (22) that hydroxytyramine inhibits the decarboxylase. They showed that adrenaline and noradrenaline and many other amines with a high affinity for amine oxidase inhibited the decarboxylase. In the adrenal medulla of the ox, Langemann (116) considers that the accumulation of amines might cause an inhibition of amine formation.

Oxidation by amine oxidase is probably an important mechanism of biological inactivation of hydroxytyramine, because the amine has a high affinity for amine oxidase. Since it is a catechol amine, two other mechanisms of inactivation have to be considered: oxidation by phenolase type of enzymes (including the cytochrome system) and conjugation of the phenolic groups with sulphuric or glucuronic acid.

These same three mechanisms of inactivation are of importance in the inactivation of adrenaline and noradrenaline: oxidative deamination, oxidation to adrenochrome (or noradrenochrome) and conjugation.

We do not know to what extent these three modes of enzymic attack contribute to the inactivation of the two amines *in vivo*. The existing evidence is most satisfactory for conjugation, and it is likely that conjugation will contribute greatly to the inactivation of adrenaline and other catechol amines when they are ingested orally or when they circulate in the blood for a sufficient length of time, so that conjugation can occur in intestine and liver. This is supported by experiments in which β-labelled radioactive dl-adrenaline was administered (for structure, see below) (153). When 0.04 μg. of adrenaline per g. body weight was injected intravenously in rats, no adrenaline, free or conjugated, was recovered from the urine. When 0.3 μg./g. was injected, about 6% was excreted as free, and 3% as conjugated, adrenaline. When 45 μg./g. of adrenaline were given by mouth, about 18% of the dose was recovered as conjugated adrenaline.

The action of phenolases and related enzymes on adrenaline results in the formation of adrenochrome. The same type of reaction also occurs in what is generally called the "autoxidation" of adrenaline.

Bacq (3) has recently pointed out that autoxidation of adrenaline is not a powerful factor in the inactivation of adrenaline, as the cells contain many substances which prevent autoxidation. On the other hand, he is inclined to attribute to the "phenolase" type of oxidation a major importance in the biological inactivation.

Substances which counteract the autoxidation of adrenaline also prevent the enzymic oxidation by phenolases. This can be easily demonstrated. When a solution of adrenaline is incubated in a manometric flask and a preparation of mushroom oxidase is added, oxygen consumption begins at once and a red colour develops. This indicates that oxidation products of adrenaline accumulate. If the same solution is incubated with mushroom phenolase in the presence of ascorbic acid, oxygen consumption again begins at once, but little colour develops. This observation is easily interpreted: the enzyme acts on adrenaline in the presence and absence of ascorbic acid; when ascorbic acid is present, the first oxidation product of adrenaline does not accumulate, but is immediately reduced to adrenaline by the ascorbic acid which is oxidized. Adrenaline is thus continuously regenerated until all ascorbic acid added has been oxidised to dehydro-ascorbic acid; only then will coloured oxidation products of adrenaline begin to accumulate. Similarly sulphydryl compounds protect adrenaline, and it is likely that all these substances exert their action also *in vivo*.

It is not necessary here to discuss the question of the chemical structure of the first oxidation product of adrenaline; it is sufficient to say that experimental evidence tells us that a reversible oxidation precedes the irreversible oxidation to adrenochrome. This may explain observations of Ruiz-Gijon who has described a compound, adrenoerythrine (127a, 148), which is approximately as active as adrenaline itself when tested for pressor or hypoglycemic action. If this compound is identical with the first oxidation product of the phenolase oxidation, we would not be surprised to find that it is reduced to adrenaline in the organism. It must be emphasized, however, that this interpretation is not accepted by Ruiz-Gijon.

Since the phenolase type of oxidation is catalysed by the cytochrome system which is almost ubiquitous in animal tissues it is surprising that there exists no evidence for formation of adrenochrome and related substances. From what has been said, the formation of adrenochrome in the living organism will require conditions in which substances like ascorbic acid and sulphydryl compounds fail to protect adrenaline from irreversible oxidation. Such a "disinhibition" of the oxidizing mechanism will only occur when the protecting substances are absent (or fully oxidized). It can be hoped that experiments with "labelled" adrenochrome (153) may eventually tell us something about this type of inactivation of adrenaline-like compounds.

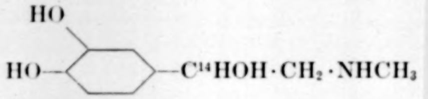
It may be added that the reversible oxidation of adrenaline to the first oxidation product is likely to occur much more readily in the tissues than the irreversible oxidation to the adrenochrome stage.

The oxidation of adrenaline and noradrenaline by amine oxidase was discovered when it became possible to exclude the "phenolase" type of oxidation by the use of cyanide (33). Since then the contribution of amine oxidase to the inactivation of adrenaline and sympathin has been under discussion.

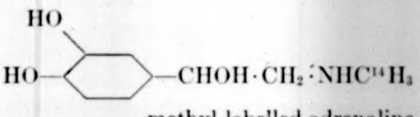
Until recently, chemical methods were not sufficiently sensitive to allow studies of the metabolic fate of adrenaline and noradrenaline. Two recent investigations will be discussed which suggest that amine oxidase is active on these two amines.

Lund (123) has followed the rate of disappearance of adrenaline and noradrenaline from the circulating blood in the rabbit and from the perfusion fluid in isolated rabbit's organs, the liver and the hind legs. In the liver the rate of disappearance of both amines was much higher than in the hind legs. The concentration of amines in these experiments was very high; it would be interesting to study the disappearance of adrenaline in lower concentration with an even more sensitive method (see, *e.g.*, 178a). Lund discusses the possibility that deamination and conjugation are responsible for the high rate of inactivation in the liver.

The second type of experiment makes use of radioactive tracer technique. Schayer (152, 153) synthesized two preparations of adrenaline which contain the radioactive isotope C^{14}. One of these has a "labelled" carbon atom in position β to the methylamino group; in the other the N-methyl carbon is labelled:



β-labelled adrenaline



methyl-labelled adrenaline

If these preparations are given intravenously or by mouth, the radioactive carbon atoms suffer different fates. About 90% of the β-carbon atom is excreted in the urine, but in the same time, only about 50–60% of the methyl carbon atom is excreted. This indicates that at least 30–40% of the adrenaline molecules are split between the β carbon and the methyl carbon atoms. Two reactions could bring this about: demethylation or oxidative deamination. The β-labelled adrenaline gives rise to a radioactive fraction in the urine which can be extracted with ether after acidification. No radioactive fraction of this kind is present when methyl-labelled adrenaline is given. These observations are compatible with the idea that some of the adrenaline had been metabolised by amine oxidase. It seems that with the help of this method we may learn to what extent the various pathways contribute to the breakdown of adrenaline and noradrenaline administered in small amounts.

Gaddum and Kwiatkowski first interpreted the potentiating action of ephedrine as due to the inhibitory action on amine oxidase (73). Schapira (150) and Thompson and Tickner (173) have since found amine oxidase in the rabbit's ear, and the possibility that amine oxidase might be active in removing adrenaline or sympathin in peripheral tissues has to be considered.

Many amines which have an affinity for amine oxidase are more or less perfect sympathicomimetics. This is true for inhibitors and substrates, not only of the phenylethylamine or phenyl*iso*propylamine series, but also for aliphatic amines like amylamine, even for long-chain diamines such as hexadecamethylene diamine $H_2N(CH_2)_{16}NH_2$. Yet, it seems by no means certain that all amines with affinity for amine oxidase are sympathicomimetic; dodecamethylene diamine is oxidized even more rapidly by amine oxidase than the C_{16} diamine, but no sympathicomimetic properties have been reported for this compound.

In order to account for the connexion between affinity for amine oxidase and sympathicomimetic action one can either assume that the sympathicomimetic effect is produced by an increase in sympathin concentration in the neighbourhood of the nerve ending; this could be brought about by inhibition of an enzyme which destroys sympathin; or the parallelism can be explained by a similarity in structure between the tissue receptors which are sensitive to sympathin, and the part of the enzyme which specifically combines with the substrate.

It is not known if an enzyme is responsible for the inactivation of sympathin at adrenergic nerve endings. The strict temporal and spatial limitation of the stimulus which is required in cholinergic nerves may not be necessary in the adrenergic system. However, the wide distribution of amine oxidase in structures innervated by adrenergic nerves makes it worth while to discuss some of the facts which point to a connexion between sympathicomimetic effect and affinity for amine oxidase.

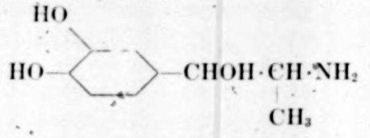
We may distinguish "direct" sympathicomimetic effects from "indirect" ones. The former are effects exerted upon the tissue receptors for sympathin; the latter are effects exerted by preserving sympathin in the neighbourhood of the excitable structure.

An "indirect" sympathicomimetic effect can only occur when there is sympathin present in the neighbourhood of the nerve endings. There are indications that the amount of sympathin present after denervation is greatly reduced (63, 64); this indicates that less or no sympathin is released. The tissue receptors for sympathin remain excitable after denervation. It should therefore be possible to use the postganglionically-denervated organ in order to distinguish the direct action of a substance on the tissue receptors from its indirect action. If no sympathin is released, the indirect action would no longer be present.

Some time ago, Burn and Tainter (44) showed that after the section of the postganglionic sympathetic nerves the cat's pupil became very insensitive to both tyramine and ephedrine, whereas the sensitivity to adrenaline was increased. Later it was shown that tyramine and ephedrine had very little action in the sympathetically-denervated fore limb of the cat. In the perfused hind limb of the dog the two amines gradually lost much of their vasoconstrictor action;

this could be restored when adrenaline was added to the perfusion fluid (40). In another set of experiments, Bülbring and Burn (38) found that the denervated nictitating membrane of the cat, although more sensitive to small doses of tyramine, responded with less powerful contractions than the normal nictitating membrane as the dose of tyramine was increased; with high doses the contractions of the normal nictitating membrane were much stronger than those of the denervated membrane.

Observations like these show that the denervated organ may prove useful in the study of "direct" actions of sympathicomimetic substances on the receptor protein; they do not allow us to conclude that the indirect actions are brought about by an inhibition of amine oxidase. Other inactivating mechanisms may be present. von Euler (63) reports that solutions of adrenaline and noradrenaline acquire a red colour when incubated with nervous tissue or spleen. He discusses the possibility that a phenolase type of enzyme may occur in these tissues. The inhibition of phenolase might explain observations on corbasil.



Corbasil

This amine has a vasoconstrictor action on the blood vessels of the rabbit's ear, which resembles that of adrenaline. Ephedrine sensitizes the vessels of the rabbit's ear to corbasil (106), yet the amine is not oxidised by amine oxidase. Corbasil is a substrate of phenolases, and an inhibition of phenolase by ephedrine would explain this observation. Nothing is known to suggest that ephedrine has such an inhibitory action.

The normally innervated nictitating membrane of the cat is more sensitive to adrenaline than to noradrenaline. After denervation the membrane becomes equally sensitive to both substances, and this is brought about by a larger increase in sensitivity to noradrenaline (39). Burn interprets this phenomenon as caused by a reduction in amine oxidase in the neighbourhood of the nerve endings: the membrane is normally less sensitive to injected noradrenaline, because the amine oxidase acts more rapidly on noradrenaline, the sympathetic mediator. The evidence which supports this interpretation has recently been reviewed by Burn (41). Sympathetic denervation is followed by a reduction in the amine oxidase of the membrane; after 10–12 days the total enzymic activity had fallen to about 66 per cent of that of the normal membrane (43). A correlation was found between reduction of enzyme and sensitization to noradrenaline: the sensitization was most marked in the membranes in which the enzymic activity had been most strongly reduced.

An observation by West (184) may be quoted in support of this interpretation: methylene blue renders the nictitating membrane more sensitive to noradrenaline, so that it resembles the denervated organ in its sensitivity.

Amine oxidase as an enzyme designed to destroy noradrenaline at sympathetic nerve endings would account for these results. But some facts, already discussed, are not easily reconciled with this picture. One is the very high concentration of enzyme in organs like the liver and kidneys. We must assume that in these tissues the enzyme has some other function. Another difficulty is in the specificity pattern of the enzyme: substances like hydroxytyramine or tyramine are more rapidly oxidised by amine oxidase than is noradrenaline and they probably have a higher affinity than amines with a hydroxyl group in β-position. We have no indication that in tissues with adrenergic innervation the enzyme may be specifically adapted to sympathin, although such a possibility has been discussed by Bernheim and Bernheim (8).

Cocaine has long been known to sensitise the tissues to the actions of adrenaline (71), and this has been explained by the inhibiting action of cocaine on amine oxidase (130). In the frog's heart, the sensitisation by cocaine to sympathetic stimulation was shown not to be due to an increased release of sympathin (46). It has been reported that in the rabbit's ear cocaine increases the amount of sympathin which comes out in the perfusate, and that it increases the amount of adrenaline recovered from the perfusate (119, 175).

In the normally innervated nictitating membrane cocaine has a stronger sensitising effect on the response to noradrenaline than on that to adrenaline; as a result, the nictitating membrane after cocaine resembles the denervated membrane (41; see also 104). All these actions of cocaine could be interpreted by assuming that cocaine acts by inhibiting the mechanism which inactivates sympathin.

However, cocaine has also some actions which are not so easily interpreted on the basis of enzyme inhibition. It antagonises the action of tyramine (171). If the action of tyramine is mainly an indirect one, it is not surprising that cocaine has no potentiating effect on tyramine, but it is surprising that the action of tyramine should be abolished.

Many other substances have affinity for amine oxidase and have also either sympathicomimetic or sympathicolytic actions. Jang (107) finds that the action of ergotoxine upon the response to adrenaline is similar in principle to that of cocaine. Tickner has recently discussed the possibility that certain antihistaminics owe their sympathicomimetic side actions to their inhibitory effects on amine oxidase (174). Schuler has examined the inhibition of amine oxidase by a number of imidazoline derivatives; he finds that the compounds with marked sympathicomimetic or sympathicolytic action are the stronger inhibitors of the enzyme. More observations could be quoted which might be considered to support the idea that inhibition of amine oxidase may play a part in the pharmacological action of sympathicomimetic compounds. However, the alternative explanation, based on a similarity of receptor protein and enzyme protein, has not satisfactorily been excluded.

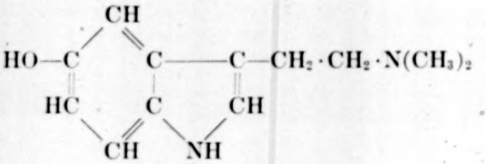
In conclusion, we can say that the organ deprived of sympathin seems suited for the study of the direct sympathicomimetic actions of a compounds. Secondly, an indirect action of a compound may be due to an inhibition of a mechanism other than amine oxidase; the phenolase type of enzyme and its inhibitors have not been systematically studied. Thirdly, a compound may have an affinity for more than one structure in the tissues; Bacq (3) considers it as certain that ephedrine acts directly on the receptor protein and Gaddum (72) has pointed out that this is not necessarily in disagreement with the assumption that some actions of ephedrine are indirect.

Derivatives of tryptamine

It has long been known that tryptamine is a substrate of amine oxidase (34, 135). The amine had never been found in the body, but an enzyme that decarboxylates the amino-acid tryptophan is said to occur in mammalian liver (180). Observations on tryptamine metabolism in animals reviewed in the historical introduction show that the amine is metabolised by oxidative deamination. The possibility that tryptamine may be converted to indoleacetic acid in plants has already been discussed.

Derivatives of tryptamine have been isolated from animal tissues and the possibility arises that amine oxidase is active in the metabolism of these substances.

Bufotenine. Bufotenine was found by Phisalix and Bertrand in the secretion of the skin glands of the toad. Jensen and Chen (108) suggested that it was an indole derivative, and this was proved by Wieland, Konz and Mittasch (185) who showed that bufotenine was N-dimethyl-5-hydroxytryptamine:



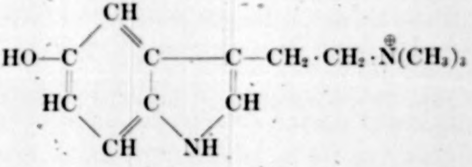
Bufotenine

The pharmacological properties of the pure substance have been studied by Raymond-Hamet (139, 140, 141) and by Erspamer (53). Bufotenine has pressor action which is enhanced by cocaine and antagonized by yohimbine. The pressor action is believed to be nicotine-like rather than adrenaline-like, because very little vasoconstrictor action can be demonstrated on the isolated perfused paw. The isolated rat's uterus, especially after treatment with oestrogens, is sensitive to bufotenine: contraction was observed in concentrations down to 2×10^{-8}.

Bufotenine is destroyed when it is incubated in an atmosphere of oxygen with extracts from tissues rich in amine oxidase: guinea-pig's liver, kidneys and small intestine. The inactivation is not affected by cyanide, but it is inhibited by β-phenyl*iso*propylamine and by methylene blue. Preparations of histaminase do not act on bufotenine.

These observations strongly suggest that the inactivation of bufotenine is brought about by an amine oxidase type of enzyme. The data given do not allow a comparison with the rate of oxidation of other amines. Bufotenine is a tertiary amine, and these are usually oxidised rather slowly by the guinea-pig enzyme.

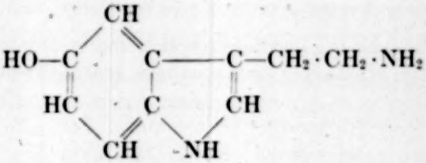
According to Erspamer (53), the quaternary base, bufotenidine:



Bufotenidine

is not inactivated by amine oxidase. This is in agreement with what is known of the properties of the enzyme.

5-Hydroxytryptamine. It now appears established that the primary amine corresponding to bufotenine is also present in extracts from animal tissues.



5-hydroxytryptamine
(Enteramine, Serotonin)

The vasoconstrictor principle present in cattle serum was first obtained as a crystalline substance in 1948 (137). It was then shown that this material contained creatinine sulphate together with an indole derivative probably identical with 5-hydroxytryptamine (138).

It has been shown that preparations from sheep lung which oxidised both tyramine and tryptamine also inactivated the serum vasoconstrictor substance (36).

The rate of oxidation of a sample of serotonin creatinine sulphate by preparations containing amine oxidase has recently been measured and compared with the rate of oxidation of tyramine and tryptamine (16). All three amines were rapidly oxidised; there was not very much difference in the rate of oxidation. Two preparations were used: a homogenate of guinea-pig's liver and one of the posterior salivary glands from *Octopus vulgaris*. It is known that tryptamine gives rise to a dark pigment when it is incubated with amine oxidase (135), and it was found that serotonin also forms a pigment.

In 1937, Vialli and Erspamer described a substance which they named enteramine. The substance was first characterized by a number of colour reactions, and has recently been identified as 5-hydroxytryptamine (59). Enteramine has been found in vertebrates and in invertebrates. In vertebrates, it occurs in the intestine, where it is believed to be the secretion product of the enterochromaffin cells, and also in the skin of some amphibians (61). In invertebrates, the posterior salivary glands of *Octopus* are a particularly rich source (55); it also occurs in the hypobranchial organ of *Murex* (57) and in the gastrointestinal tract of *Ascidia*, *e.g.*, *Ciona intestinalis* (54).

Like bufotenine, enteramine has a marked excitatory effect upon the rat's uterus in oestrus. It also has an antidiuretic action on the hydrated rat; this is due to a constrictor action on the afferent glomerular vessels.

There are indications that enteramine from natural sources is a substrate of amine oxidase (51, 52). The "enteraminase" described by Erspamer is present in many animal tissues; it is inhibited by β-phenyl*iso*propylamine, by methylene blue and octyl alcohol, but not by cyanide. Enteramine from the posterior salivary glands of *Octopus vulgaris* and of *Eledone moschata* was also destroyed by preparations from guinea-pig's liver (55).

A sample of synthetic 5-hydroxytryptamine picrate has recently been tested as a substrate of amine oxidase. As expected, the amine was found to be rapidly oxidised by preparations of guinea-pig's liver and of the posterior salivary glands of *Octopus vulgaris* (16). In other words, the synthetic amine was oxidised like serotonin.

These observations raise the question whether the inactivation of 5-hydroxytryptamine is a normal function of amine oxidase. It would be necessary to show that 5-hydroxytryptamine is a normal tissue constituent. Erspamer considers enteramine as the secretion product of the enterochromaffine system and he stresses the hormone character of the amine. If this is so, then the removal of the amine may be a normal function of amine oxidase in the tissues. The strong action of enteramine on the afferent glomerular vessels might explain why the kidneys are so rich in amine oxidase.

The occurrence of 5-hydroxytryptamine raises the question: how is it formed? The very weak action on tryptophan of the decarboxylase described by Werle and Menniken (180) might be explained if the natural substrate of this enzyme in the body were not tryptophan but 5-hydroxytryptophan.

SUMMARY

Amine oxidase is an enzyme system that catalyses the oxidative deamination of many amines.

The enzyme is widely distributed in the animal kingdom and possibly in plants. In vertebrates, it occurs particularly in glandular tissue, in plain muscle and in the nervous system.

The enzyme is bound to the cytoplasmatic particles, mitochondria and microsomes.

Although amine oxidase reacts with oxygen *in vitro*, the possibility is discussed that in the living tissue the enzyme does not directly react with molecular oxygen.

The amines react with the enzyme when they are in the ionised state; amino compounds which are feebly ionised at the pH of the tissues are not oxidised.

The study of the substrate specificity of the enzyme for aliphatic amines shows that the reaction between amino group and the enzyme takes place only if there is also some hydrophobic group present in the substrate molecule. This is believed to be necessary for the attachment and orientation of the substrate molecule on the enzyme surface.

A review of the metabolism of amines not normally present in the body shows that many of these are metabolised as would be expected if the amine oxidase reaction occurs in the body. Many amines which are not substrates of the enzyme are not destroyed in the body. The substrate specificity for amine oxidase is therefore an important factor in determining the duration of action of many amino compounds.

Possible substrates of the enzyme in the living organism are discussed. Of the sympathicomimetic amines which occur in mammals, hydroxytyramine may be an important substrate; the question of the oxidation of noradrenaline and adrenaline is connected with the problem of sympathicomimetic action of substances with affinity for amine oxidase (*e.g.*, tyramine, β-phenyl*iso*propylamine and its derivatives, cocaine). Reasons for and against the role of the enzyme as an agent that inactivates sympathin in the neighbourhood of adrenergic nerve endings are discussed.

Amine oxidase acts rapidly on 5-hydroxytryptamine; if it can be shown that this amine occurs regularly in the body, its inactivation may well be an important function of amine oxidase.

REFERENCES

1. Alles, G. A., and Heegaard, E. V.: Substrate specificity of amine oxidase. J. Biol. Chem., **147:** 487-503, 1943.
2. Axelrod, A. E., Sober, H. A., and Elvehjem, C. A.: The d-amino acid oxidase content of rat tissues in riboflavin deficiency. J. Biol. Chem., **134:** 749-759, 1940.
3. Bacq, Z. M.: The metabolism of adrenaline. Pharmacol. Rev., **1:** 1-26, 1949.
4. Barger, G., and Dale, H. H.: Chemical structure and sympathomimetic action of amines. J. Physiol., **41:** 19-59, 1910.
5. Bernhard, K.: Zur Spaltung der Amidbindung in Tierkörper. II. Über den Abbau von Propylamin und Amiden der Kork- und Sebacinsäure. Hoppe-Seyl. Z., **256:** 65-70, 1938.
6. Bernheim, F.: The effect of propamidine on bacterial metabolism. Science, **98:** 223, 1943.
7. Bernheim, F., and Bernheim, M. L. C.: The oxidation of mescaline and certain other amines. J. Biol. Chem., **123:** 317-326, 1938.
8. Bernheim, F. and Bernheim, M. L. C.: The inactivation of tyramine by heart muscle in vitro. J. Biol. Chem., **158:** 425-431, 1945.
9. Bernheim, M. L. C.: Tyramine oxidase II. The course of the oxidation. J. Biol. Chem., **93:** 299-309, 1931.
10. Beyer, K. H.: Sympathomimetic amines: the relation of structure to their action and inactivation. Physiol. Rev., **26:** 169-197, 1946.
11. Beyer, K. H., and Govier, W. M.: The deamination of "marfanil" and related compounds. Science, **101:** 150-151, 1945.
12. Beyer, K. H., and Morrison, H. S.: Relation of structure to deamination of sympathomimetic amines. Indust. & Engin. Chem., **37:** 143-148, 1945.
13. Beyer, K. H., and Skinner, J. T.: The detoxication and excretion of beta phenylisopropylamine (benzedrine). J. Pharmacol., & Exper. Therap. **68:** 419-432, 1940.
14. Bhagvat, K., Blaschko, H., and Richter, D.: Amine oxidase. Biochem. J., **33:** 1338-1341, 1939.
15. Birkhäuser, H.: Fermente im Gehirn geistig normaler Menschen. Helv. chim. Acta, **23:** 1071-1086, 1940.
16. Blaschko, H.: Unpublished observations.
17. Blaschko, H.: Amine oxidase and ephedrine. J. Physiol., **93:** 7P, 1938.
18. Blaschko, H.: Amine oxidase and diamines. The action of guanidine. J. Physiol., **95:** 30P, 1939.
19. Blaschko, H.: The specific action of l-dopa decarboxylase. J. Physiol., **96:** 50P-51P, 1939.
20. Blaschko, H.: Amine oxidase and benzedrine. Nature, London, **145:** 26, 1940.
21. Blaschko, H.: Amine oxidase in Sepia officinalis. J. Physiol., **99:** 364-369, 1941.
22. Blaschko, H.: The activity of l-dopa decarboxylase. J. Physiol., **101:** 337-349, 1942.
23. Blaschko, H.: Enzymic oxidation of mescaline in the rabbit's liver. J. Physiol., **103:** 13P-14P, 1944.
24. Blaschko, H.: Adrenaline and sympathin. In: The Hormones. Physiology, Chemistry and Applications. Vol. II, p. 601. Academic Press Inc., New York, 1950.
25. Blaschko, H. and Burn, J. H.: Oxidation of adrenaline and noradrenaline by amine oxidase. J. Physiol., **112:** 37P, 1951.
26. Blaschko, H., Burn, J. H., and Langemann, H.: The formation of noradrenaline from dihydroxyphenylserine. Brit. J. Pharmacol., **5:** 431-437, 1950.
27. Blaschko, H., and Duthie, R.: The inhibition of amine oxidase by amidines. Biochem. J., **39:** 347-350, 1945.
28. Blaschko, H., and Duthie, R.: Substrate specificity of amine oxidases. Biochem. J., **39:** 478-481, 1945.
29. Blaschko, H. and Hawkins, J.: Enzymic oxidation of aliphatic diamines. Brit. J. Pharmacol., **5:** 625-632, 1950.

30. Blaschko, H., and Hawkins, J.: Amine oxidase in cephalopods. J. Physiol., **118:** 88–93, 1952.

31. Blaschko, H., Holton, P., and Sloane Stanley, G. H.: Enzymic formation of pressor amines. J. Physiol., **108:** 427–439, 1949.

32. Blaschko, H., and Philpot, F. J.: Unpublished observations.

33. Blaschko, H., Richter, D., and Schlossmann, H.: The inactivation of adrenaline. J. Physiol., **90:** 1–17, 1937.

34. Blaschko, H., Richter, D., and Schlossmann, H.: The oxidation of adrenaline and other amines. Biochem. J., **31:** 2187–2196, 1937.

34a. Blaschko, H., Richter, D., and Schlossmann, H.: Enzymic oxidation of amines. J. Physiol., **91:** 13P–14P, 1937.

35. Bovet, D., and Bovet-Nitti, F.: Structure et activité pharmacodynamique des medicaments du système nerveux végétatif. Bâle, S. Karger, 1948.

36. Bradley, T. R., Butterworth, R. F., Reid, G., and Trautner, E. M.: Nature of the lung enzyme which inactivates serum vasoconstrictor. Nature, London, **166:** 911–912, 1950.

37. Brodie, A. F., and Gots, J. S.: Effects of an isolated dehydrogenase enzyme and flavoprotein on the reduction of triphenyltetrazolium chloride. Science, **114:** 40–41, 1951.

38. Bülbring, E., and Burn, J. H.: The action of tryamine and adrenaline on the denervated nictitating membrane. J. Physiol., **91:** 459–473, 1938.

39. Bülbring, E., and Burn, J. H.: Liberation of noradrenaline from the suprarenal gland. Brit. J. Pharmacol., **4:** 202–208, 1949.

40. Burn, J. H.: The action of tyramine and ephedrine. J. Pharmacol. & Exper. Therap., **46:** 75–95, 1932.

41. Burn, J. H.: The enzyme at sympathetic nerve endings. Brit. M. J., 1952 (i): 784–787, 1952.

42. Burn, J. H., and Robinson, J.: Noradrenaline and adrenaline in vessels of the rabbit ear in relation to the action of amine oxidase. Brit. J. Pharmacol., **6:** 101–109, 1951.

43. Burn, J. H., and Robinson, J.: Effect of denervation on amine oxidase in the nictitating membrane. J. Physiol., **116:** 21P–22P, 1952.

44. Burn, J. H., and Tainter, M. L.: An analysis of the effect of cocaine on the actions of adrenaline and tryamine. J. Physiol., **71:** 169–193, 1931.

45. Chen, K. K., Wu, C.-K., and Henriksen, E.: Relationship between the pharmacological action and the chemical constitution and configuration of the optical isomers of ephedrine and related compounds. J. Pharmacol. & Exper. Therap., **36:** 363–400, 1929.

46. Clark, A. J., and Raventos, J.: Response of tissues to sympathetic stimulation. Quart. J. Exper. Physiol., **29:** 165–183, 1939.

47. Cotzias, G. C., and Dole, V. P.: Metabolism of amines. I. Microdetermination of monoamine oxidase in tissues. J. Biol. Chem., **190:** 665–672, 1951.

48. Cotzias, G. C., and Dole, V. P.: Metabolism of amines. II. Mitochondrial localization of monoamine oxidase. Proc. Soc. Exper. Biol. & Med., **78:** 157–160, 1951.

49. Easson, L. H., and Stedman, E.: The absolute activity of cholinesterase. Proc. Roy. Soc., **B121:** 142–164, 1936.

50. Epps, H. M. R.: The development of amine oxidase activity by human tissues after birth. Biochem. J., **39:** 37–42, 1945.

51. Erspamer, V.: Pharmakologische Studien über Enteramin. IV Mitteilung: Über die Inaktivierung von Enteramin durch tierisches Gewebe. Arch. f. exper. Path. u. Pharmakol., **200:** 43–59, 1942.

52. Erspamer, V.: Pharmakologische Studien über Enteramin. VI Mitteilung: Weitere Untersuchungen uber die Inaktivierung von Enteramin durch tierisches Gewebe. Arch. f. exper. Path. u. Pharmacol., **201:** 377–390, 1943.

53. Erspamer, V.: Ricerche farmacologiche sulle indolalchilamine del veleno di rospo. I. Bufotenina e bufotenidina. Arch. Sci. biol., **31:** 63–84, 1946.

54. Erspamer, V.: Presenza di enteramina o di una sostanza enteraminosimile negli estratti gastrointestinali e splenici dei pesci e negli estratti gastroenterici delle Ascidie. Experientia, **2:** 369–371, 1946.

55. Erspamer, V.: Active substances in the posterior salivary glands of Octopoda. I. Enteramine-like substance. Acta pharmacol. et toxicol., **4:** 213–223, 1948.

56. Erspamer, V.: Active substances in the posterior salivary glands of Octopoda. II. Tyramine and octopamine. Acta pharmacol. et toxicol., **4:** 224–247, 1948.

57. Erspamer, V.: Ricerche chimiche e farmacologiche di Murex trunculus, Murex brandaris e Tritonalia erinacea. IV. Presenza negli estratti di enteramina o di una sostanza enteraminosimile. Arch. internat. de pharmacodyn., et de thérap. **76:** 308–326, 1948.

58. Erspamer, V.: Identification of octopamine as l-p-hydroxyphenylethanolamine. Nature, London, **169:** 375–376, 1952.

59. Erspamer, V., and Asero, B.: Identification of enteramine, the specific hormone of the enterochromaffin cell system, as 5-hydroxytryptamine. Nature, London, **169:** 800–801, 1952.

60. Erspamer, V., and Boretti, G.: Identification and characterization, by paper chromatography, of enteramine, octopamine, tyramine, histamine and allied substances in extracts of posterior salivary glands of Octopoda and in other tissue extracts of vertebrates and invertebrates. Arch. internat. de pharmacodyn., et de thérap., **88:** 296–332, 1951.

61. Erspamer, V., and Vialli, M.: Presence of enteramine in the skin of amphibia. Nature, London, **167:** 1033, 1951.

62. von Euler, U. S.: Noradrenaline (arterenol), adrenal medullary hormone and chemical transmitter of adrenergic nerves. Ergebn. d. Physiol., **46:** 261–307, 1950.

63. von Euler, U. S.: The nature of adrenergic nerve mediators. Pharmacol. Rev., **3:** 247–277, 1951.

64. von Euler, U. S., and Purkhold, A.: Effect of sympathetic denervation on the noradrenaline content of the spleen, kidney and salivary glands in the sheep. Acta physiol. Scandinav., **24:** 212–217, 1952.

65. **Ewins, A. J.,** and **Laidlaw, P. P.**: The fate of p-hydroxyphenylethylamine in the organism. J. Physiol., **41**: 78–87, 1910.

66. **Ewins, A. J.,** and **Laidlaw, P. P.**: The fate of indolethylamine in the organism. Biochem. J., **7**: 18–25, 1913.

67. **Fastier, F. N.,** and **Hawkins, J.**: Inhibition of amine oxidase by isothiourea derivatives. Brit. J. Pharmacol., **6**: 256–262, 1951.

68. **Fellows, E. J.,** and **Bernheim, F.**: The effect of a number of arylalkylamines on the oxidation of tyramine by amine oxidase. J. Pharmacol. & Exper. Therap., **100**: 94–99, 1950.

69. **Florence, G.,** and **Schapira, G.**: Sur une méthode de caractérisation de l'amine-oxydase. Compt. rend. Soc. biol., Paris, **139**: 35–36, 1945.

70. **Friedenwald, J. S.,** and **Herrmann, H.**: The inactivation of amine oxidase by enzymatic oxidative products of catechol and adrenaline. J. Biol. Chem., **146**: 411–419, 1942.

71. **Fröhlich, A.,** and **Loewi, O.**: Uber eine Steigung der Adrenalinempfindlichkeit durch Cocain. Arch. f. exper. Path. u. Pharmakol., **62**: 159–169, 1910.

72. **Gaddum, J. H.**: The action of local hormones. Proc. Roy. Soc., **B137**: 292–297, 1950.

73. **Gaddum, J. H.,** and **Kwiatkowski, H.**: The action of ephedrine. J. Physiol., **94**: 87–100, 1938.

74. **Gale, E. F.**: Factors influencing the enzymic activities of bacteria. Bact. Rev., **7**: 140–173, 1943.

75. **Gale, E. F.**: The bacterial amino acid decarboxylases. Advances Enzymol., **6**: 1–32, 1946.

76. **Gaskell, J. F.**: The chromaffine system of annelids and the relation of the system to the contractile vascular system in the leech, *Hirudo medicinalis*. A contribution to the comparative physiology of the contractile vascular system and its regulators, the adrenalin secreting system and the sympathetic nervous system. Philos. Trans. *B*, **205**: 153–211, 1914.

77. **Gaskell, J. F.**: Adrenalin in annelids. A contribution to the comparative study of the origin of the sympathetic and the adrenalin-secreting systems and of the vascular muscles which they innervate. J. Gen. Physiol., **2**: 73–85, 1919/1920.

78. **Ghiretti, F.**: Personal communication.

79. **Gonnard, P.**: Action d'amines cycliques et acycliques sur la dopadécarboxylation. Bull. Soc. chim. biol., **32**: 535–540, 1950.

80. **Goodall, McC.**: Studies of adrenaline and noradrenaline in mammalian hearts and suprarenals. Acta physiol. Scandinav., **24**: Suppl. 85, 1951.

81. **Govier, W. M., Grelis, M. E., Yanz, N. S.,** and **Beyer, K. H.**: Studies on the mechanism of action of sympathomimetic amines. III. The oxidation of tyramine by rat liver homogenates. J. Pharmacol. & Exper. Therap., **87**: 149–158, 1946.

82. **Gregerman, R. I.**: Adrenaline and hydroxytyramine in the parotid gland venom of the toad, *Bufo marinus*. J. Gen. Phsyiol., **35**: 483–487, 1952.

83. **Grewal, R. S.,** and **Blaschko, H.** Unpublished observations.

84. **Guggenheim, M.**: Die biogenen Amine und ihre Bedeutung für die Physiologie und Pathologie des pflanzlichen und tierischen Stoffwechsels. 4th Ed. Basel and New York: S. Karger, 1951.

85. **Guggenheim, M.,** and **Löffler, W.**: Das Schicksal proteinogener Amine im Tierkörper. Biochem. Ztschr., **72**: 325–350, 1916.

86. **Hare, M. L. C.**: Tyramine oxidase. I. A new enzyme system in liver. Biochem. J., **22**: 968–979, 1928

87. **Harris, S. C., Searle, L. M.,** and **Ivy, A. C.**: The excretion of amphetamine. J. Pharmacol. & Exper. Therap., **89**: 92–96, 1947.

88. **Hartles, R. L.,** and **Williams, R. T.**: The metabolism of sulphonamides. 4. The relation of the metabolic fate of Ambacide (Marfanil) and V 335 to their lack of systemic antibacterial activity. Biochem. J., **41**: 206–218, 1947.

89. **Hartles, R. L.,** and **Williams, R. T.**: A study of the relation between conjugation and deamination of p-hydroxybenzylamine and related compounds. Biochem. J., **43**: 296–303, 1948.

90. **Hartung, W. H.**: Inactivation and detoxication of pressor amines. Ann. Rev. Biochem., **15**: 593–611, 1946

91. **Hawkins, J.**: The localization of amine oxidase in the liver cell. Biochem. J., **50**: 577–581, 1952.

92. **Hawkins, J.**: Amine oxidase activity of rat liver in riboflavin deficiency. Biochem. J., **51**: 399–404, 1952.

93. **Hawkins, J.,** and **Walker, J. M.**: The effect of colchicine on the enzyme content of regenerating rat liver and on the pressor amine content of the adrenal. Brit. J. Pharmacol., **7**: 152–160, 1952.

94. **Heegaard, E. V.,** and **Alles, G. A.**: Inhibitor specificity of amine oxidase. J. Biol. Chem., **147**: 505–513, 1943.

95. **Heim, F.**: Die Bedeutung der experimentellen Änderung der Aminoxydase-Aktivität für die Adrenalinwirkung. Arch. f exper. Path. u. Pharmakol., **204**: 520–542, 1947.

96. **Heim, F.**: Uber den Einfluss von Alkoholen auf den enzymatischen Abbau des Tyramins. Arch. f exper. Path. u. Pharmakol., **210**: 16–22, 1950.

97. **Heim, F.**: Uber den Einfluss verschiedener Ester auf die fermentative Oxydation von Adrenalin und Adrenalinderivaten. Arch. f exper. Path. u. Pharmakol., **209**: 181–193, 1950.

98. **Heim, F.** and **Koelle, G.**: Uber Beziehungen zwischen der Dauer der aminoxydase-hemmenden Wirkung von Novocain, Pantokain und Anästhesin und der Geschwingigkeit ihres enzymatischen Abbaus durch Leber und Niere. Arch. f exper. Path. u. Pharmakol., **211**: 303–312, 1950.

99. **Heim, F.,** and **Reim, R.**: Die Wirkung des Xanthins und mehrerer methylierter Xanthinderivate auf die Aktivität der spezifischen Cholinesterase und Aminoxydase. Arch. f. exper. Path. u. Pharmakol., **207**: 703–710, 1949.

100. **Henze, M.**: p-Oxyphenyläthylamin, das Speicheldrüsengift der Cephalopoden. Hoppe-Seyl. Z., **87**: 51–58, 1913.

101. **Holtz, P., Credner, K.,** and **Reinhold, A.**: Aminbildung durch Darm. Arch. f. exper. Path. u. Pharmakol., **193**: 688–692, 1939.

102. **Holtz, P., Credner, K.,** and **Strübing, C.**: Uber das Vorkommem der Dopadecarboxylase im Pancreas. Arch. f. exper. Path. u. Pharmakol., **199**: 145–152, 1942.

103. Holtz, P., Heise, R., and Lüdtke, K.: Fermentativer Abbau von l-Dioxyphenylalanin (DOPA) durch Niere. Arch. f. exper. Path. u. Pharmakol., **191:** 87–118, 1938.

104. Innes, I. R., and Kosterlitz, H. W.: The effect of cocaine and chronic sympathetic denervation of the heart on the chronotropic action of adrenaline and noradrenaline. Brit. J. Pharmacol., **6:** 651–658, 1951.

105. Jacobsen, E., and Gad, I.: Das Verhalten des β-Phenylisopropylamins im Kaninchenorganismus. II. Abbau, Organverteilung und Ausscheidung bei normalen Tieren. Arch. f. exper. Path. u. Pharmakol., **196:** 34–42, 1940.

105a. Jacobsen, E., and Gad, I.: Die Ausscheidung des β-phenylisopropylamins bei Menschen. Arch. f. exper. Path. u. Pharmakol., **196:** 280–289, 1940.

106. Jang, C. S.: Interaction of sympathicomimetic substances on adrenergic transmission. J. Pharmacol., & Exper. Therap. **70:** 347–361, 1940.

107. Jang, C. S.: The potentiation and paralysis of adrenergic effects by ergotoxine and other substances. J. Pharmacol. & Exper. Therap., **71:** 87–94, 1941.

108. Jensen, H., and Chen, K. K.: Chemische Studien über Kröten Gifte. V Mitteil.: Die basischen Bestandteile des, Kröten-Sekrets. Ber. dtsch. chem. Ges., **65:** 1310–1314, 1932.

109. Keilin, D., and Hartree, E. F.: Coupled oxidation of alcohol. Proc. Roy. Soc. *B*, **119:** 141–159, 1936.

110. Kenten, R. H., and Mann, P. J. G.: Oxidation of amines by pea seedlings. Biochem. J., **50:** 360–369, 1952.

111. Klevens, H. B.: Critical micelle concentrations as determined by refraction. J. Phys. Colloid Chem., **52:** 130–148, 1948.

112. Kohn, H. I.: Tyramine oxidase. Biochem. J., **31:** 1693–1704, 1937.

113. Krebs, H. A.: The D- and L-amino acid oxidases. Biochem. Soc. Symp., **1:** 2–18, 1948.

114. Kuhn, R., and Jerchel, D.: Über Invertseifen. VIII. Reduktion von Tetrazoliumsalzen durch Bakterien. Ber. dtsch. chem. Ges., **74B:** 949–952, 1941

115. Langemann, H.: Weitere Untersuchungen über das Vorkommen der Cholinesterase und der Monoaminoxydase in menschlichen Organen. 3. Mitteilung über Bestimmungen der Fermentaktivität in normalen und pathologischen Organen. Helv. Physiol. Acta, **2:** 367–375, 1944.

116. Langemann, H.: Enzymes and their substrates in the adrenal gland of the ox. Brit. J. Pharmacol., **6:** 318–324 1951.

117. Langemann, H., Roulet, F., and Zeller, E. A.: Fermentuntersuchungen an gesunden und tuberkulösen menschlichen Lungen. Klin. Wchnschr., **22:** 42–43, 1943.

118. Larsen, V.: Das Verhalten des β-Phenylisopropylamins im Kaninchenorganismus. III. Der Abbau des β-Phenylisopropylamins in überlebenden Kaninchenorganen. Arch. f exper. Path. u. Pharmakol., **196:** 43–50, 1940.

119. Lawrence, W. S., Morton, M. C., and Tainter, M. L.: Effects of cocaine and sympathomimetic amines on humoral transmission of sympathetic nerve actions. J. Pharmacol. & Exper. Therapy., **75:** 219–225, 1942.

120. Leaf, G., and Neuberger, A.: The preparation of homogentisic acid and of 2:5-dihydroxyphenylalanine. Biochem. J., **43:** 606–610, 1948.

121. Lee, H. M., and Chen, K. K.: The occurrence of norepinephrine in the Chinese toad venom. J. Pharmacol. & Exper. Therap., **102:** 286–290, 1951.

122. Löffler, W.: Desaminierung und Harnstoffbildung im Tierkörper. Biochem. Ztschr. **85:** 230–294, 1918,

123. Lund, A.: Elimination of adrenaline and noradrenaline from the organism. Acta pharmacol. et toxicol., **7:** 297–308, 1951.

124. Luschinsky, H. L., and Singher, H. O.: Identification and assay of monoamine oxidase in the human placenta. Arch. Biochem.. **19:** 95–107, 1948.

125. Mann, P. J. G., and Quastel, J. H.: Benzedrine (β-phenylisopropylamine) and brain metabolism. Biochem. J. **34:** 414–431, 1940.

126. Minkowski, O.: Uber Spaltungen im Thierkörper. Arch f exper. Path. u. Pharmakol., **17:** 445–465, 1883.

127. Mosso, U.: Quantitative Untersuchungen über die Ausscheidung der Salicylsäure und der Umwandlungsprodukte des Benzylamins aus dem thierischen Organismus. Arch. f exper. Path. u. Pharmakol., **26:** 267–278, 1889.

127a. Muracciole, J. C., and Ruiz-Gijon, J.: Etude comparée de l'action qu'exercent sur la glycémie l'adrénaline, l'adrénoérythrine, l'adrénochrome et le monohydrate de 3:5:6-trihydroxy-1-méthylindol. Arch. internat. de physiol., **59:** 265–272, 1951.

128. Orzechowski, G.: Uber die Wirkungsweise der Sympathikomimetika. IX. Die stoffliche Blockade spezifischer Grenzflächen als Ursache der Tachyphylaxie der Sympathikomimetika. Beeinflussung der Adrenalinoxydase. Arch. f exper. Path. u. Pharmakol., **198:** 27–33, 1941.

129. Philpot, F. J.: Some observations on the oxidation of tyramine in the liver. Biochem. J., **31:** 856–861, 1937.

130. Philpot, F. J.: The inhibition of adrenaline oxidation by local anaesthetics. J. Physiol., **97:** 301–307, 1940.

131. Philpot, F. J., and Cantoni, G.: Adrenaline destruction and methylene blue. J. Pharmacol. & Exper. Therap. **71:** 95–103, 1941.

132. Poll, H., and Sommer, A.: Uber phaeochrome Zellen im Centralnervensystem des Blutegels. Arch. Anat. Physiol., **1903:** 549–550.

133. Polonovski, M., Schapira, G., and Gonnard, P.: Constitution chimique et inhibition enzymatique; dopa-décarboxylase et amines inhibitrices. Rapports entre dopa-décarboxylase et amine-oxydase. Bull. Soc. Chim. Biol., **28:** 735–739, 1946.

134. Pugh, C. E. M., and Quastel, J. H.: Oxidation of aliphatic amines by brain and other tissues. Biochem. J., **31:** 286–291, 1937.

135. Pugh, C. E. M., and Quastel, J. H.: Oxidation of amines by animal tissues. Biochem. J., **31:** 2306–2321, 1937.

136. Randall, L. O.: Oxidation of phenethylamine derivatives by amine oxidase. J. Pharmacol. & Exper. Therap. **83:** 216–220, 1946.

137. Rapport, M. M.: Serum vasoconstrictor (serotonin). V. The presence of creatinine in the complex. A proposed structure of the vasoconstrictor principle. J. Biol. Chem., **180:** 961–969, 1949.

138. Rapport, M. M., Green, A. A., and Page, I. H.: Serum vasoconstrictor (serotonin). IV. Isolation and characterization. J. Biol. Chem., **176:** 1243–1251, 1948.

139. Raymond-Hamet: Sur l'action vasculaire locale de la bufoténine. Compt. rend. Soc. biol., Paris, **135:** 1414–1416, 1941.

140. Raymond-Hamet: Sur les effets vasculaires de la bufoténine introduite dans la circulation générale. Compt. rend. Acad. sc., Paris, **214:** 506–508, 1942.

141. Raymond-Hamet: Sur le mécanisme de l'action vasoconstrictrice de la bufoténine. Compt. rend. Acad. sc. Paris, **214:** 687–688, 1942.

142. Rechenberger, J.: Über die flüchtigen Alkylamine im menschlichen Stoffwechsel. II. Mitteilung: Ausscheidung im Harn nach oraler Zufuhr. Hoppe-Seyl. Z., **265:** 275–284, 1940.

143. Richter, D.: Adrenaline and amine oxidase. Biochem. J., **31:** 2022–2028, 1937.

144. Richter, D.: Elimination of amines in man. Biochem. J., **32:** 1763–1769, 1938.

145. Robinson, J.: Amine oxidase in the iris and nictitating membrane of the cat and the rabbit. Brit. J. Pharmacol., **7:** 99–102, 1952.

146. Robinson, R.: Synthesis in biochemistry. J. chem. Soc., **1936:** 1079–1090.

147. Rossiter, R. J.: Some factors influencing the oxidation of alanine by liver tissue. J. Biol. Chem., **135:** 431–436, 1940.

148. Ruiz-Gijon, J.: Preparation and biological activity of adrenoerythrine (?-adrenalinquinone). Nature, London, **166:** 831–832, 1950.

149. Rutenburg, A. M., Gofstein, R., and Seligmann, A. M.: Preparation of new tetrazolium salt which yields blue pigment on reduction and its use in demonstration of enzymes in normal and neoplastic tissues. Cancer Research, **10:** 113–121, 1950.

150. Schapira, G.: Répartition de l'amine-oxydase et métabolisme de l'adrénaline. Compt. rend. Soc. biol., Paris, **139:** 36–37, 1945.

151. Schapira, G.: Dopa-décarboxylase et adrénaline. Compt. rend. Soc. biol., Paris, **140:** 173–174, 1946.

152. Schayer, R. W.: Studies of the metabolism of β-C^{14}-dl-adrenalin. J. Biol. Chem., **189:** 301–306, 1951.

153. Schayer, R. W.: The metabolism of adrenalin containing isotopic carbon. J. Biol. Chem., **192:** 875–881, 1951.

154. Schmalfuss, H., and Heider, A.: Tyramin und Oxytyramin, blutdrucksteigernde Schwarzvorstufen des Besenginsters, *Sarothamnus scoparis Link*. Biochem. Ztschr., **236:** 226–230, 1931.

155. Schmiedeberg, O.: Über das Verhältniss des Ammoniaks und der primären Monoaminbasen zur Harstoffbildung im Thierkörper. Arch. f exper. Path. u. Pharmakol., **8:** 1–14, 1877.

156. Schmiedeberg, O.: Über Spaltungen und Synthesen im Thierkörper. Arch. f exper. Path. u. Pharmakol., **14:** 379–392, 1881.

157. Schofield, B. M.: Unpublished observation.

158. Schöpf, C., and Bayerle, C.: Zur Frage der Biogenese der Isochinolin-alkaloide. Die Synthese des 1-Methyl-6:7 dioxy-1:2:3:4-tetrahydroisochinolins unter physiologischen Bedingungen. Ann. Chem., **513:** 190–202, 1934.

159. Schuler, W.; quoted from R. Meier: Antistine and related imidazolines. Ann. New York Acad. Sc., **50:** 1161–1176 1950.

160. Schwarzenbach, G.: Zur Berechnung intramolekularer Atomabstände aus den Dissociationskonstanten zweibasischer Säuren. II. Die Acidität einiger Dicarbonsäuren und Polymethylendiammoniumionen in Wasser-Alkohol-Misschungen. Helv. Chim. Acta, **16:** 522–533, 1933.

161. Sereni, E.: The chromatophores of cephalopods. Biol. Bull., Woods Hole, **59:** 247–268, 1930.

162. Sinclair, H. M.: Pyridoxal phosphate as coenzyme of histaminase. Biochem. J., **51:** X–XI, 1952.

163. Singer, J. P., and Barron, E. S. C.: Studies on biological oxidations. XX. Sulfydryl enzymes in fat and protein metabolism. J. Biol. Chem., **157:** 241–253, 1945.

164. Sloane Stanley, G. H.: Unpublished observations.

165. Slotta, K. H., and Müller, J.: Über den Abbau des Mescalins und mescalinähnlicher Stoffe im Organismus. Hoppe-Seyl. Z., **238:** 14–22, 1936.

166. Snyder, F. H., Goetze, H., and Oberst, F. W.: Metabolic studies on derivatives of β-phenylethylamine. J. Pharmacol. & Exper. Therap., **86:** 145–150, 1946.

167. Snyder, F. H., and Oberst, F. W.: Metabolic studies on β-cyclohexylethylamines. J. Pharmacol. & Exper. Therap., **87:** 389–391, 1946.

168. Späth, E., and Berger, F.: Über eine für die Phytochemie bemerkenswerte Synthese des d,l-Tetrahydropapaverins. Ber. dtsch. chem. Ges., **63:** 2098–2102, 1930.

169. Spinks, A.: Unpublished observation.

170. Steensholt, G.: On an amine oxidase in rabbit's liver. Acta physiol. Scandinav., **14:** 356–362, 1947.

171. Tainter, M. L., and Chang, D. K.: The antagonism of the pressor action of tyramine by cocaine. J. Pharmacol. & Exper. Therap., **30:** 193–207, 1927.

172. Thompson, R. H. S., and Tickner, A.: Observations on the mono-amine oxidase activity of placenta and uterus. Biochem. J., **45:** 125–130, 1949.

173. Thompson, R. H. S., and Tickner, A.: The occurrence and distribution of mono-amine oxidase in blood vessels. J. Physiol., **115:** 34–40, 1951.

174. Tickner, A.: Inhibition of amine oxidase by antihistamine compounds and related drugs. Brit. J. Pharmacol., **6:** 606–610, 1951.

175. Torda, C.: Effect of cocaine and the inactivation of epinephrine and sympathin. J. Pharmacol. & Exper. Therap., **78:** 331–335, 1943.

176. Walker, J.: Some observations on salts of amidines and related compounds. J. chem. Soc., **1949:** 1996–2002.

177. Warburg, O.: Uber sauerstoffatmende Körnchen aus Leberzellen und über Sauerstoffatmung in Berkefeld-Filtraten wässriger Leberextracte. Pflügers Arch., **154:** 599–617, 1913.

178. Warburg, O.: Wasserstoffübertragende Fermente. Berlin: W. Sänger, 1948.

178a. Weil-Malherbe, H., and Bone, A. D.: The chemical estimation of adrenaline-like substances in blood. Biochem. J., **51:** 311–318. 1952.

179. Werle, E., and Boden, W.: Zur Frage des Vorkommens von Aminosäurendecarboxylasen, sowie der Histaminase, Tyramin- und Tryptaminoxydase in pflanzlichen Material, sowie über eine blutdrucksteigernde Substanz in Hefe. Biochem. Ztschr., **304:** 371–376, 1940.

180. Werle, E., and Menniken, G.: Uber die Bildung von Tryptamin aus Tryptophan und von Tyramin aus Tyrosin durch tierische Gewebe. Biochem. Ztschr., **291:** 325–327, 1937.

181. Werle, E., and v. Pechmann, E.: Uber die Diaminoxydase der Pflanzen und ihre adaptive Bildung durch Bakterien. Liebigs Ann., **562:** 44–60, 1949.

182. Werle, E., and Röwer, F.: Monoaminoxydase in Pflanzen. Biochem. Ztschr. **320:** 298–301, 1950.

183. Werle, E., and Röwer, F.: Uber tierische und pflanzliche Monoaminoxydasen. Biochem. Ztschr., **322:** 320–326, 1952.

184. West, G. B.: Methylene blue and amine oxidase. J. Physiol., **113:** 8P, 1951.

185. Wieland, H., Konz, W., and Mittasch, H.: Die Konstitution von Bufotenin und Bufotenidin. Uber Krötengiftstoffe VII. Liebigs Ann. d. Chemie, **513:** 1–25, 1934.

186. Winterstein, E., and Trier, G.: Die Alkaloide. 1st Ed. Berlin, 1910.

187. Zeller, E. A.: Diamin-oxydase. Advances Enzymol., **2:** 93–112, 1942.

188. Zeller, E. A.: Oxidation of amines. In: The Enzymes. Chemistry and Mechanism of Action. Edited by J. B. Sumner and K. Myrbäck. Vol. II, Part 1: 536–558, Academic Press Inc., New York, 1951.

189. Zeller, E. A., and Joel, C. A.: Beiträge zur Fermentchemie des männlichen Geschlechtsapparates. 2. Mitt.: Uber das Vorkommen der Cholinesterase der Mono- und Diaminoxydase in Sperma und Prostata, und über die Beeinflussung der Spermienbeweglichkeit durch Fermentinhibitoren. Helv. chim. Acta, **24:** 968–976, 1941.

190. Zeller, E. A., Stern, R., and Wenk, M.: Uber die Diamin-Diamin-oyxdase Reaktion. Helv. chim. Acta, **23:** 3–17, 1940.

"The meat and substance of the physiological education of countless young physicians"

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